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RESTING RETICULO-ENDOTHELIAL CELLS AND THEIR "FLUID VACUOLES": REACTIONS TO POTASSIUM, TONICITY AND ALBUMIN[1, 2]

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The conception of these studies developed during investigations of quite different concern. It had been noted during observations concerned with the suitability of bovine plasma albumin as a substitute for homologous serum in examination of living tissues (23) that the "resting-wandering cells," or histiocytes, of normal connective tissues were rounder and smaller when moistened with dilutions of the albumin in Locke's solution than when moistened with serum. Actually, the "resting-wandering cells" had become macrophages. This effect was puzzling, in view of the spreading action of the solution on the other tissues studied, until it was found that it also occurred with Locke's solution alone. The conversion of these cells to forms characteristic of the macrophages of inflammation was recognized as a moot point in hematology, and search for the explanation of this action of Locke's solution was therefore undertaken.

Due to the confusion of terms used for the members of the reticulo-endothelial system it is best to define the terms to be used in this contribution at this point. The term "resting-wandering cell" is used synonymously with that of clasmatocyte, as coined by Ranvier (17), or of histiocyte, as coined by Kiyono (7). It is used in the sense defined by its originator, Maximow (9, 10), to designate the large, extended, phagocytic mononuclears which occur as more or less fixed forms in normal subcutaneous tissues, and which store vital and supravital dyes avidly. Use of the term, however, is limited entirely to designation of a cytologic type, and is without commitment, whatever, to the concepts of its author concerning its origin or fate.

The term monocyte is used as defined by Sabin and co-workers (19, 20) to designate the large phagocytic mononuclear cells of the blood. It is inclusive of the two older terms devised by Ehrlich, namely large mononuclears and transitionals. Monocytes may also occur normally in blood forming organs (5, 21) and connective tissues (20).

The term macrophages is used as defined by Metchnikoff (12) to designate

[1] This work was carried out under U. S. Atomic Energy Commission Contract AT(30-1)-901 with the New England Deaconess Hospital, Boston, Massachusetts.

[2] These data have been presented in abstract before the American Association of Anatomists, Anatomical Record, **112**: 397, 1952.

the free mononuclear phagocytes found in inflammatory reactions and in parenchymatous organs. It is synonymous with the term polyblast as used by Maximow (9). Other than these, none of the synonymous terms for each type of cell will be employed.

The numerous polemics concerned with the characteristics and interrelations of the various mononuclear cells, phagocytic or otherwise, of the connective tissues and blood forming organs, which abounded during the first thirty years of this century, served to elucidate the morphology and many of the functions of these cells under various experimental or pathologic conditions, and to add vital and supravital methods of study to those already existent. None-the-less they left indefinite some of the most pressing questions concerning the hematologic and reticulo-endothelial systems, and the underlying uncertainties left from that period still continue to plague and confuse all efforts at order or simplification of medical knowledge in these fields. Understanding of etiology, diagnosis and treatment must, perforce, remain confused until definitive answers to these fundamental questions can be brought into agreement by workers with various techniques.

One of the questions which has remained moot concerns the source of the influx of macrophages which characterizes inflammation. The school using fixed techniques, and represented most widely, perhaps, by the concepts of Maximow and his associates (9), contends that they arise by two processes: 1, influx of small mononuclears from the blood stream that grow to the stature of macrophages, and 2, rounding and mobilization of the pre-existing clasmatocytes, or "resting-wandering cells", originally present in the area of the inflammation. As that school also believes that the macrophages from hematogenous sources ultimately become "resting-wandering cells," the origin of all macrophages, according to these views, would therefore, be hematogenous. The school supported by supravital studies, on the other hand, believing in the integrity of the monocyte as a separate type of cell, rather than as a functional stage in the cycle of phagocytic mononuclears, considers that the pre-existing clasmatocytes, or "resting-wandering cells", of the connective tissues are stimulated in inflammation either to hypertrophy, or to division into smaller daughter cells, the latter giving rise to the macrophages of inflammation (20). Students of this school have since conceded the probability that monocytes develop into macrophages in inflammation (5, 22), but have not, heretofore, reported the phenomenon of direct conversion of pre-existent "resting-wandering cells" into similar forms. The group supported by various methods of vital staining (4, 11) derive the macrophages of inflammation entirely from local sources, i.e., from endothelial cells and from mobilization of pre-existing histiocytes, the latter having also arisen originally from endothelium. These opposing views have been discussed by Lewis (8). His findings with tissue

cultures largely support the concepts of those working with fixed tissues in inflammation.

The findings to be reported now corroborate, by means of supravital techniques, the contention based upon fixed techniques that the "resting-wandering cells" of the subcutaneous tissue can metamorphose into macrophages. They demonstrate, furthermore: 1, that this can occur in the absence of inflammation, and without preceding division; 2, that in so doing the cells shorten and round and exhibit changes in their fluid content which suggest that they share actively in the extra-intracellular exchange of fluids in the subcutaneous tissues; and 3, that this metamorphosis is initiated by potassium.

MATERIALS AND METHODS

Male mice, C3H strain and about six months of age, were anesthetized with pentobarbital sodium given intraperitoneally in physiologic saline and exsanguinated by cardiac puncture in order to avoid extravasation at the experimental sites. The serum was separated for succeeding experiments. Upon cessation of respiration pinhead sized bits of subcutaneous tissue were excised from the back and abdomen and quickly filmed onto #0 coverslips. The films were immediately covered with the experimental fluid under study, superimposed on glass slides, rimmed with vaseline and placed either in a hot box (37°C.) containing the microscope, or in an ice chest (about 4.5°C.) until desired. Those in the hot box were observed soon after preparation. The others were left in the ice chest for various intervals up to four hours and were removed to the hot box as desired. Observation of all films usually began within ten to twenty minutes of removal to the hot box.

Serum covered films were used as controls for each mouse. The fluids used to moisten the experimental films include: 1, a phosphate buffered solution[3] related to Locke's solution; 2, Locke's solution; 3, Locke's solution from which the potassium chloride was omitted; 4, the same with and without albumin (Armour's crystalline, bovine, plasma albumin); 5, Locke's solution with increased amounts of potassium chloride; 6, Locke's solution of decreased or increased tonicity by means of shifts in the content of sodium chloride; and 7, Locke's solution diluted with homologous serum. The data concerning the ingredients and physical properties of the Locke's solution and of the phosphate buffered solution are given in Table I.

All fluids used for moistening were stained with neutral red shortly before use. This was attained by adding the fluid to serology tubes containing standard amounts of crystalline neutral red. The tubes had been previously prepared by addition of appropriate aliquots of a dilute alcoholic solution of neutral red followed by evaporation over a steam bath. Double the amount was needed with serum as with Locke's or other saline solutions. This held true whether or not albumin was added. 0.268 mg of neutral red to 1 cc. of serum was necessary for satisfactory staining.

The films were counted differentially as soon as adequately stained. Only appropriately thin and uninjured areas were counted. Areas containing mast cells were excluded. Otherwise classification included all cells present, i.e., fibroblasts, any hematogenous forms, and clasmatocytes, or "resting-wandering cells." The latter were subdivided into those which were normally expanded, i.e., true "resting-wandering cells," and those which were contracted. Contraction varied from partial to complete rounding, but only those cells which

[3] See foot note to Table I.

showed unquestionable evidence of retraction were classified as contracted. The ratio of contracted to expanded cells was computed. Each count included 100–200 cells. Varying numbers of films were counted from each area of each animal, and varying numbers of animals were used for each stage of the experiment.

The significant data under the various experimental conditions are summarized in Table II. The table indicates the number of mice used and the total number of films counted in any given group of experiments, together with the average ratio of contracted to normally expanded "resting-wandering cells." The fibroblasts suffered no consistent morphologic changes incident to any of the experimental procedures except where the solution exerted lethal effects on all cells. They have, therefore, not been tabulated, although included in the counts. The incidence of hematogenous cells in normal subcutaneous tissues of mice is negligible, and their chance presence in no way influences the ratio between contracted and extended "resting-wandering cells." Therefore, they also have been excluded from the table. The average values for these exclusions are discussed briefly below.

TABLE I

*Ingredients and Properties of Locke's and Phosphate Buffered Solutions**

SOLUTION	GRAMS PER 1000 CC OF SOLUTION									PHYSICAL PROPERTIES	
	NaCl	KCl	$NaHCO_3$	Phosphates		$MgCl_2$	$MnCl_2$	Dextrose	$CaCl_2$	pH	Freezing point
				NaH_2	Na_2H						
Locke's solution 1.	9.0	0.42	0.30	—	—	—	—	1.00	0.24	6.35	−0.69
Locke's solution 2.	9.0	0.42	0.30	—	—	—	—	1.00	0.24	7.62	−0.64
Phosphate buffered...	3.94	0.41	—	1.28	6.25	0.10	0.002	1.00	0.06	7.3	−0.54

* We are very grateful to Dr. Ralph W. McKee, Division of Biological Chemistry, this Institute, for the phosphate buffered solution and for the determinations of pH and freezing point included in this table.

FINDINGS

I. Control films moistened with homologous serum (Table II, column 1)

The "resting-wandering cells," or clasmatocytes, are uniformly expanded and similar in all films covered with serum (Figures 1 and 2). Contracted, or rounded forms are practically negligible. The ratio of contracted to expanded cells is 0.1. The normal, expanded cells present interesting characteristics. They tend to occur in clusters, side by side, or end on end. The cytoplasm is filmed over wide distances with considerable branching, but always with definite margins that are very thin and clear. The nucleus is small, about half the size of that of the fibroblast, and darker gray than in the latter, homogenous, or with one faint nucleolus, and of low refractivity. It tends to be centrally placed, or at one side of the main body of the cells from which the various cytoplasmic expansions take origin. Angular, yellowish, refractive

bodies of about a micron in length, are usual in the main body of the cell, and these are usually birefringent and slowly take on a brown hue in the presence of neutral red. In addition to these there occur many phagocytic vacuoles in the main body of the cells. These vary in size from less than one to several microns, stain apricot to dark red, and are rarely birefringent. Droplets of fat are rare. The expanded cytoplasm beyond these various deposits is veil-like in transparency, and colorless. The expansions contain many clear spaces but rarely any dye. It is only slowly that these spaces take on faint amounts of the dye,

TABLE II

*Contractile Action of Different Moistening Agents upon "Resting-Wandering Cells"**

COLUMN	MOISTENING AGENT	TOTAL NUMBER MICE USED	TOTAL NUMBER FILMS COUNTED	AVERAGE RATIO, CONTRACTED CELLS/ EXPANDED CELLS
1	*Homologous Serum*	22	71	0.1
	Locke's Solution			
2	Solution 1	6	23	1.0
3	Solution 2	12	49	1.2
4	Albumin added—0.75%	4	15	1.3
5	—1.50%	5	15	1.3
6	—4.00%	2	8	0.7
7	—6.00%	3	8	1.6
	K omitted			
8	Na normal	7	24	0.1
9	Albumin added—2.00%	2	9	0.1
10	Na balanced	2	16	0.2
11	K normal, Na decreased	4	14	0.7
	K increased, Na balanced			
12	K—125% normal	1	3	1.0
13	—150% normal	2	11	2.4
14	—200% normal	1	5	2.9
15	*Phosphate Buffered Solution*	3	13	1.1
16	Albumin added—1.50%	4	20	1.5

* The word "normal" in this table is used to connote the same amount of a given ion as called for by the standard formula for Locke's solution (Table I). The word "balanced" is used to connote change in the number of sodium ions called for in the standard formula to such amount as to balance the experimental modification of the number of potassium ions.

and then they become a chalky pink which is quite different from the color in the phagocytic vacuoles described above. The term "fluid vacuoles" is used to designate these spaces. These "resting-wandering cells" are, apparently, well anchored. They show no signs of movement. No undulations of the cytoplasmic expansions are detectable.

The average percentage of fibroblasts and of reticulo-endothelial cells, based on the counts of 346 separate films was 67.4 and 26.4, respectively. These values were obtained from both the control and experimental counts, and

include all reticulo-endothelial cells, irrespective of whether they were expanded or contracted. The remaining 6.2% in the average counts includes sporadic clusters of eosinophils, and stray neutrophils and lymphocytes.

II. Films moistened with Locke's solutions (Table I; Table, II, columns 2 and 3)

Both solutions used for these studies were hypertonic compared with blood. They varied considerably from each other in pH. When first examined, the "resting-wandering cells" moistened with either solution appeared somewhat indrawn and shortened, but otherwise like those in the control films covered with serum. This was true whether the films had been placed directly in the hot box or held back in the ice chest. Staining of the phagocytic vacuoles was pale, but its intensity increased a at normal rate of speed. The "fluid vacuoles" were not stained. Within a short period of beginning examination, however, these cells began to show metamorphoses which ultimately terminated in complete retraction of the cytoplasmic expansions, together with shortening and rounding of the cells, development of zones of dense, yellow ectoplasm, and partial to complete disappearance of the "fluid vacuoles." These changes began within ten minutes of placing freshly made films in the hot box, and at slightly longer intervals if the films had been stored in the ice chest before removal to the hot box. The sequence of events could be followed best, actually, with the latter films, since the process had been held in abeyance in the cold and could be followed more slowly as the films warmed. As said, at first the films appeared very much as described in serum except that their contact with the dye was too brief for completed staining of the phagocytic vacuoles. Gradually the cytoplasmic expansions became slightly denser, broadened at their tips and began to retract. As this occurred the "fluid vacuoles" seemed to shrink, or recede. Contraction occurred in all cytoplasmic expansions simultaneously so that the body of the cell broadened, became denser, and gradually assumed oval to round shapes (Figure 3). During this period the phagocytic vacuoles within the body of the cell darkened in color, but remained close to the nucleus, optically sharp and with the same variance in size and color as when the cells are exposed to serum. Many of these contracting cells terminated as free macrophages with large amounts of neutral red and with narrow rims of fluffy, or still vacuolated, ectoplasm, which remained normally transparent and colorless (Figure 3). If vacuoles were present they were in all respects like the "fluid vacuoles" in the "resting-wandering cells" of the control films except that they were smaller. Others of these cells contracted more markedly until they became surrounded by broad bands of dense, yellowish ectoplasm devoid of "fluid vacuoles" (Figure 6). In this extreme the cells became thicker, and the optical definition of both cytoplasm and phagocytic vacuoles lessened, giving the impression of a certain degree of edema. The nuclei did not stain, however, the

phagocytic vacuoles remained unchanged in other respects, and all evidence pointed to continued viability. The ratio of contracted to expanded cells reached unity and above. Whether shortened but still containing "fluid vacuoles," or more tightly contracted with only a band of dense ectoplasm, devoid of "fluid vacuoles", these contracted cells were similar to the macrophages that may be found free in the organs of normal animals, or that appear in great numbers in areas of inflammation, except for the droplets of fat which appear in macrophages in inflammation. These macrophages developed, while under observation, simply by slow contraction of the cytoplasmic expansions that had anchored the "resting-wandering cells," together with more or less loss, or shifting, of the fluid in the large vacuoles of the expansions. The process from inception to cessation at either stage of contraction required from ten to thirty minutes. Sometimes long threads of cytoplasm extended from various points of the periphery at stages in this contraction (Figures 4 and 5). They were highly refractive and gave the cells the appearance of centipedes or many legged spiders. They did not contain vacuoles and were narrow and more like fibers than cytoplasm. They belonged with the cytoplasm of the body of the cell, however, as they eventually became incorporated within the general layer of ectoplasm of the end stages.

III. Films moistened with phosphate buffered solution (Table I; Table II, column 15)

The pH and tonicity of this solution agreed more closely with those of blood than was true of the Locke's solutions, and they were subject to surer control during experimental use. The glucose was added separately before use. The proportions of the common ingredients varied somewhat from those of Locke's solution except in the case of the potassium chloride. This was the same for both solutions. This solution did not contain the bicarbonate used to buffer Locke's solution, but employed the phosphate buffers instead. It also contained magnesium and manganese in addition to the ingredients contained in Locke's solution. It was chosen in order to control the possibility that the contractile action of Locke's solution might be concerned with its hypertonicity or pH.

The "resting-wandering cells" in films moistened with this phosphate buffered solution reacted similarly to those in the films moistened with either batch of Locke's solution. Shrinkage of the "fluid vacuoles" was, on the whole, less extreme than in the films covered with the Locke's solutions, but it was definite, none-the-less, compared to the status in the control films covered with serum. The ratios of contracted to expanded cells were in the same range as in films covered with the Locke's solutions.

IV. Films moistened with Locke's solution the tonicity of which was decreased by means of shifts in the amount of sodium chloride (60–100% of the standard proportion) (Table II, column 9)

The "resting-wandering cells" went through the process of contraction, and the ratios of contracted to expanded cells increased, just as when exposed to the standard, somewhat hypertonic Locke's solutions. The process was neither slowed nor hastened. The only difference in the end result consisted in the degree of shrinkage of the "fluid vacuoles". The cytoplasm retracted and the cells rounded, regardless of the tonicity, but the "fluid vacuoles" varied: they were larger and more prominent in the solutions of decreased tonicity than in the isotonic, phosphate buffered solution; and they were larger in the latter than in the hypertonic, standard Locke's solutions, in which they often completely disappeared.

V. Films moistened with Locke's solution containing modified amounts of potassium chloride

a. Potassium chloride omitted (Table II, column 8). The "resting-wandering cells" did not contract. They retained the same appearance as when moistened with serum. They remained fixed in expanded positions, and the ratios of contracted to expanded cells stayed normal. The "fluid vacuoles" remained large (Figure 7). The viability of the cells equalled that when moistened with serum.

b. Potassium chloride omitted, sodium chloride balanced (Table II, column 10). The "resting-wandering cells" appeared normal in all respects. No appreciable difference was noted from their status in films moistened with serum, or from that discussed immediately above (Section V, a) (Figures 8 and 9).

c. Content of potassium chloride 200% of normal, sodium chloride balanced (Table II, column 14). This amount of potassium chloride proved lethal. The "resting-wandering cells", as well as the fibroblasts, stained diffusely, were unduly refractile, and appeared dead. They did not contract.

d. Content of potassium chloride 150% of normal, sodium chloride balanced (Table II, column 13). This amount was also lethal to the majority of the cells. Those that survived responded poorly along the lines that they respond to standard Locke's solution.

e. Content of potassium chloride 125% of normal, sodium chloride balanced (Table II, column 12). The cells responded as in standard Locke's solution.

VI. Films moistened with solutions containing albumin

The optical clarity of fibers and cells was strikingly sharpened in all films moistened with albumin. This is in agreement with findings reported elsewhere (23).

a. Locke's solution, albumin added (0.75–6.0%) (Table II, columns 4–7

inclusive). The "resting-wandering cells" responded as when moistened with Locke's solution alone.

b. Phosphate buffered solution, albumin added (*1.5%*) (Table II, column 16). The "resting-wandering cells" responded as when moistened with the buffered solution alone.

c. Potassium chloride omitted from Locke's solution, albumin added (*2.0%*) (Table II, column 9). As in the same solution before addition of albumin, the "resting-wandering cells" remained normally expanded and the ratio of contracted to expanded cells remained low. The "fluid vacuoles", however, became enormous, and the optical definition sharpened (Figures 10 and 11). The vacuoles were appreciably larger than in cells exposed to the same solution without the albumin. This was in marked contrast to the contraction and loss of "fluid vacuoles" observed when the films were moistened with the standard Locke's solution containing albumin. As noted, the "resting-wandering cells" responded in that situation exactly as when moistened with Locke's solution alone.

VII. Films moistened with half and half mixtures of standard Locke's solution and homologous serum

The "resting-wandering cells" retained the same appearance as when moistened with serum alone (Figure 12). Counts were not made.

DISCUSSION

The findings indicate that those cells of the reticulo-endothelial system which normally lie spread and fixed in the connective tissues, and to which the name "resting-wandering cells" is ascribed, are capable of retracting their cytoplasmic expansions and of becoming transformed into typical, free macrophages. It has been demonstrated by observations on intact living organisms that they are capable of this transformation in amphibian embryos (2). That similiar capacity is retained in adult, mammalian tissues has also long been believed by workers with fixed techniques (10), but this has been held in doubt by those using supravital methods of study unless division ensued first (20). The latter workers were unable to obtain evidence in their studies on inflammation of direct transformation of the expanded, quiescent cells of connective tissues into the free macrophages of the inflammatory reaction. The present observations, which were obtained by following transformations within individual cells by supravital methods, and in the absence of inflammation and of the confusing cytologic types that customarily invade areas of inflammation, represent uncomplicated evidence that "resting-wandering cells" of the adult can contract, and that they can change into macrophages. The presence of potassium, in the absence of serum, will initiate these changes. They are unaccompanied by mitosis or cellular division. Thus another of the prolonged

controversies of hematologic literature that are based upon differences of technique is brought into accord.

The high content of potassium that is usual to most cells would lead one to expect increased amounts of this ion in areas of cytologic injury. Increased amounts have been reported in areas of inflammation (6). It seems probable, therefore, that conversion of the local "resting-wandering cells" into macrophages in injured areas is under the influence of potassium liberated locally.

While conversion of the local "resting-wandering cells" into macrophages does not represent, by any means, the largest quotum of macrophages in inflammation, it evidently does represent the quotum in the early hours of inflammation before appearance of cells from hematogenous sources, or while the latter are still in process of maturation to more phagocytic stages. Similar observations were obtained earlier in studies of living connective tissue following injections of colloidal cholesterol (22). The local "resting-wandering cells" contracted and became loaded with acicular crystals of the sterol.

This action of potassium in initiating contraction of "resting-wandering cells" seems specific to the ion, and only indirectly dependent upon tonicity. Tonicity affects the degree to which the cells lose their content of "fluid vacuoles," and therefore their ultimate size; but contraction, *per se*, seems to be initiated by the potassium *per se*. It is obvious that serum must contain factors not present in tissue juice which hold this action of potassium in abeyance since the cells remain normally expanded when moistened with serum, or even with Locke's solution diluted with equal parts of serum. The inhibiting factor is obviously not albumin since the cells contract when moistened with Locke's solution containing albumin, although not when moistened with the same solution from which the potassium has been omitted. Actually, under the latter condition albumin exerts an expansive effect upon the cells and their "fluid vacuoles" similar to its action on erythrocytes and lymphocytes (23). The large vacuolar content in the cytoplasmic expansions of the "resting-wandering cells" and the slowness with which they became tinted with the supravital dye, in contrast to the speed and intensity of staining of the phagocytic vacuoles in the body of the cells close to the nuclei, are suggestive, if not indicative, of the probability that these cells contain two sorts of cytoplasmic vacuoles: one, typical of the phagocytic vacuoles that characterize all phagocytic cells, the other, more specific to these cells, and dependent upon osmotic modifications of the environment for their size and abundance. By this latter system of vacuoles the cells seem intimately linked to the rapid exchange between the intra-extra-cellular fluids. By virtue of this second vacuolar apparatus the cells are able to hold back large amounts of fluid from the extra-cellular environment, and yet, at the same time, to keep this fluid also separate from the cellular components. This retention of fluid does not represent a true edema, in which condition the cytoplasmic constituents

imbibe fluid. It represents a definite segregation of fluid within separate compartments of the cell, separate from both cytoplasmic constituents and from phagocytic vacuoles. Each of these structures within the cell retains its optical sharpness, which is quite unlike the situation in edema. While thus separated from the other cytoplasmic components, these vacuoles are evidently affected both by cytoplasmic contraction and by changes in the tonicity of the environment. They rarely disappear completely in any appreciable number of cells during contraction, provided the environment remains isotonic. But they do become smaller. No changes occur in the appearance of the phagocytic vacuoles, on the other hand, during contraction in isotonic solution; and only moderate changes occur, even with changes in tonicity within the limits of viability. The response of the "fluid vacuoles" to changes in tonicity, on the other hand, is active. They remain fairly large, even in face of contraction, provided the environment is hypotonic, and disappear almost completely if the environment is sufficiently hypertonic. The data do not permit deductions as to what may be the content of these vacuoles, if other than water, but it is logical to suppose that they are related to the so-called tissue juices and add their moieties of protein and saline to the interstitial fluids. In this sense they recall the concepts of Renaut (18) and of Pfuhl (15) who consider these cells to be of rhagiocrin nature with the capacity of secretion. The distribution and appearance of the "fluid vacuoles" throughout the cytoplasmic expansions suggest a glorified scale of the more delicate vacuolar apparatus recently found in many types of cells by Palade and Porter (13) with the use of electron microscopy. The change in hue of the cytoplasm from colorless to faint yellow during contraction is not understood.

The findings do not permit conclusions concerning real changes in volume of the "resting-wandering cells" incident to cytoplasmic contraction or to shrinkage of the "fluid vacuoles." The perimeter of the cells obviously lessens as they contract, but at the same time the cells become thicker. Pfuhl (15) discusses the fallaciousness of deductions concerning either size or color of these cells when based on area only. Ponder (16) has presented careful measurements in proof of the same fact in the case of erythrocytes, and it would seem as if his data may be regarded as significant to all cells in general. While the "fluid vacuoles" shrink, or actually disappear, in the present instance, it can not be said with certainty that they are entirely excreted. So long as the optical definition of a cell remains sharp it seems likely that the fluid is excreted rather than dissipated among the cytoplasmic components, and that volume is reduced accordingly. But when optical cloudiness accompanies the changes in the cell it seems probable that more or less fluid has been shifted from the vacuoles to the cytoplasmic components, without change of volume, but with the production of a true edema, none-the-less.

In the case of these cells, then, one can draw sharp distinctions between:

first, diffusion of fluid from either cytoplasmic vacuoles or interstitial spaces directly onto the cytoplasmic constituents with production of true edema; and, second, segregation of fluid from interstitial spaces into specific cytoplasmic compartments, i.e., "fluid vacuoles", without the production of edema in the true sense of the word. The former seems harmful to the cell, the latter a protective mechanism for conservation, as well as for removal of excess fluid. It seems probable that it is the abundant numbers of these cells (see Findings, Section 1) in a normal state of storage in subcutaneous tissues which accounts for the large proportion of total body water in the skin (14).

The metamorphoses of the "resting-wandering cells" as they contrast present striking similarities to the descriptions by Vimtrup (24) of Rouget cells about capillaries. Vimtrup believed these cells to be contractile elements related to muscle fibers. Others regard them as adventitial cells arising from the connective tissues (1, 15). The present findings might be regarded as harmonizing these contending concepts. They obviously can not be accepted as definite proof, however, since observations of capillaries were not carried out. Clark and Clark (2), on the other hand, observed rounding, followed by active migration, on the part of cells stretched along capillaries in living amphibian embryos, and presented evidence that the rounded cells were macrophages. Their observations of the connective tissues beneath windows in rabbits' ears (3) suggest similar occurrence in the adult mammal, although the evidence in this instance is incomplete. It seems highly probable that the present observations eal with identical cells to those that they observed.

CONCLUSIONS

1. The so-called "resting-wandering" cells of the connective tissue are normally expanded and filled with two types of vacuoles. Typical phagocytic vacuoles, deeply staining with neutral red, fill the body of the cell surrounding the nucleus. Large, poorly staining "fluid vacuoles" fill the cytoplasmic expansions.

2. These cells can contract and become transformed to macrophages, in the absence of inflammation or of influx of hematogenous cells, and without preceding division.

3. Contraction is initiated by solutions containing potassium in the absence of serum. This is independent of tonicity. It is inhibited by the presence of serum. It is accompanied by moderate shrinkage of the "fluid vacuoles".

4. The extent of emptying of the "fluid vacuoles," however, together with the ultimate degree of contraction of the cells, are affected by tonicity. The vacuoles remain large, and the cells therefore less contracted, in hypo- than in hypertonic environments.

5. The "fluid vacuoles" of these cells seem to represent an intracellular segregation apparatus which is separated from the interstitial fluids of the

environment, and at the same time from the phagocytic vacuoles and other components of the cells; yet which is capable of active exchange of fluids in either direction.

6. Albumin exerts an expansive or "spreading" effect upon these cells and upon their "fluid vacuoles" in the absence of potassium.

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PLATE LEGENDS

All photographs are of "resting-wandering cells" in living films of connective tissue from mice. All were taken with an oil immersion lens and 10× ocular. All magnifications are the same, ×900. The cells were large, and constantly undergoing slow changes in shape and shifts in position of the vacuoles. Sharp focus at this magnification of more than one cell of a cluster was therefore usually impossible. Frequently choice had to be made between sacrifice in detail of the other cells of a cluster for the sake of sharp focus of one, or more uniform depiction of the group at sacrifice of sharp focus of any. Phagocytic vacuoles stained with neutral red appear black in the photographs. "Fluid vacuoles" appear as pale gray areas or as spaces of variable dimensions. Cytoplasm is only faintly distinguishable by a difference in refractivity from that of the surroundings. The tracings outline the cytoplasmic margins and the fluid vacuoles. The phagocytic vacuoles are not traced. Nuclear margins are traced only in figures 2, 3, 4, 5, 9 and 11.

PLATE I

FIG. 1. Mouse no. 64. Film moistened with homologous serum. A "resting-wandering cell". It was normally expanded; phagocytic vacuoles stained with neutral red were situated close to the nucleus; and "fluid vacuoles" of various sizes filled the cytoplasmic expansions throughout their entire length. The latter are shown best in the upper end of the cell.

FIG. 2. Mouse no. 63. Film moistened with homologous serum. A group of "resting-wandering cells" which were normally expanded and touching end on end. Same comments as for Figure 1. Vacuolar detail is shown best in the bottom cell.

FIG. 3. Mouse no. 64. Film moistened with Locke's solution. A group of "resting-wandering cells" which had all contracted moderately and therefore separated from each other. The cytoplasmic expansions had retracted, the "fluid vacuoles" had decreased in number but were still plentiful, and the cells had thickened. Optical definition, however, was sharp and there were no indications of true edema. The "fluid vacuoles" are shown best in the middle cell.

FIG. 4. Mouse no. 66. Film moistened with Locke's solution. A "resting-wandering cell" in a transitional stage of contraction. The cytoplasmic expansions had lost their "fluid vacuoles" but still anchored the cell to the surrounding tissue by fine, cord-like bands which extended radially. The cell had rounded and thickened in the process.

FIG. 5. Same cell as Figure 4, ten minutes later. The cord-like strands of cytoplasm had shortened and almost entirely retracted into the body of the cell.

FIG. 6. Mouse no. 65. Film moistened with Locke's solution. A group of "resting-wandering cells" that had completed contraction. The cytoplasmic expansions had retracted to form a dense band of ectoplasm. The "fluid vacuoles" had practically vanished. The phagocytic vacuoles stained with neutral red remained normal. The cells were typical macrophages.

PLATE II

FIG. 7. Mouse no. 66. Film moistened with Locke's solution from which potassium chloride was omitted. The "resting-wandering cells" were normal in every respect. The same comments apply as for Figure 1. The "fluid vacuoles" are most distinct at the upper tip of the cell.

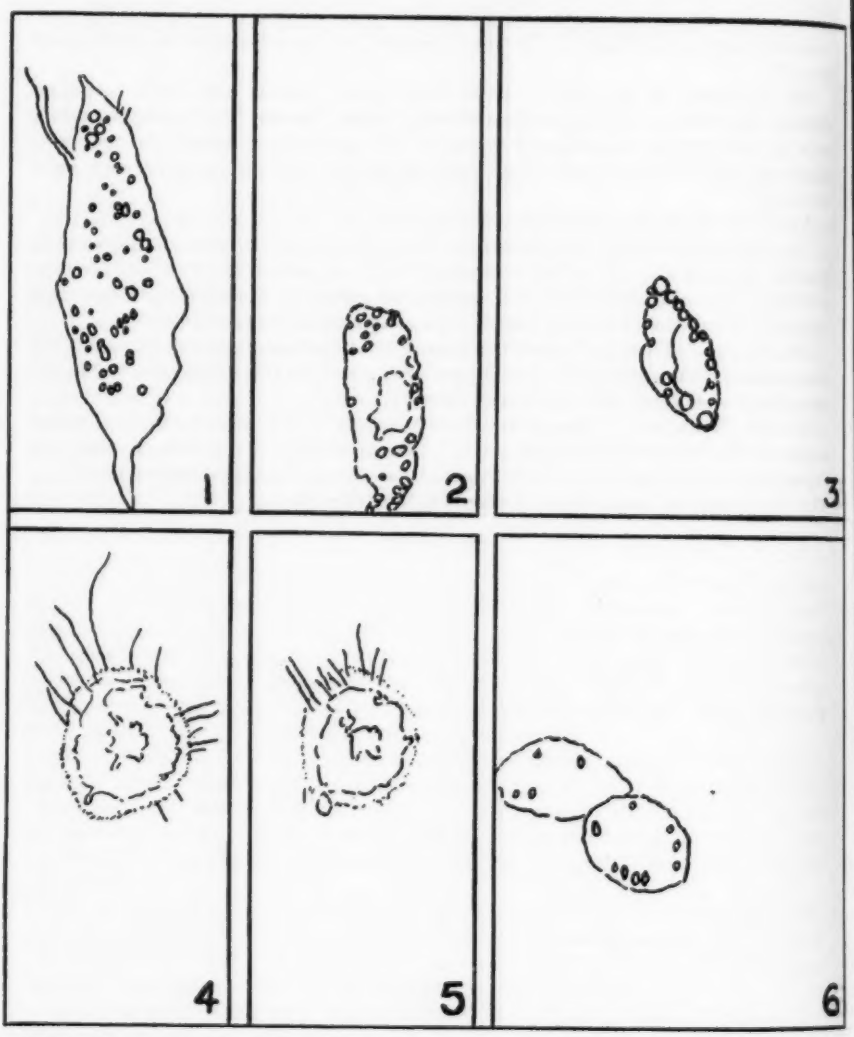
FIG. 8. Mouse no. 68. Film moistened with Locke's solution from which potassium chloride was omitted, but balanced by addition of sodium chloride. The "resting-wandering cells" did not contract and remained typical of their appearance in serum. The same comments apply as in Figures 1 and 7. The "fluid vacuoles" are most distinct at the upper right of the cell.

FIG. 9. Same film and comments as for Figure 8.

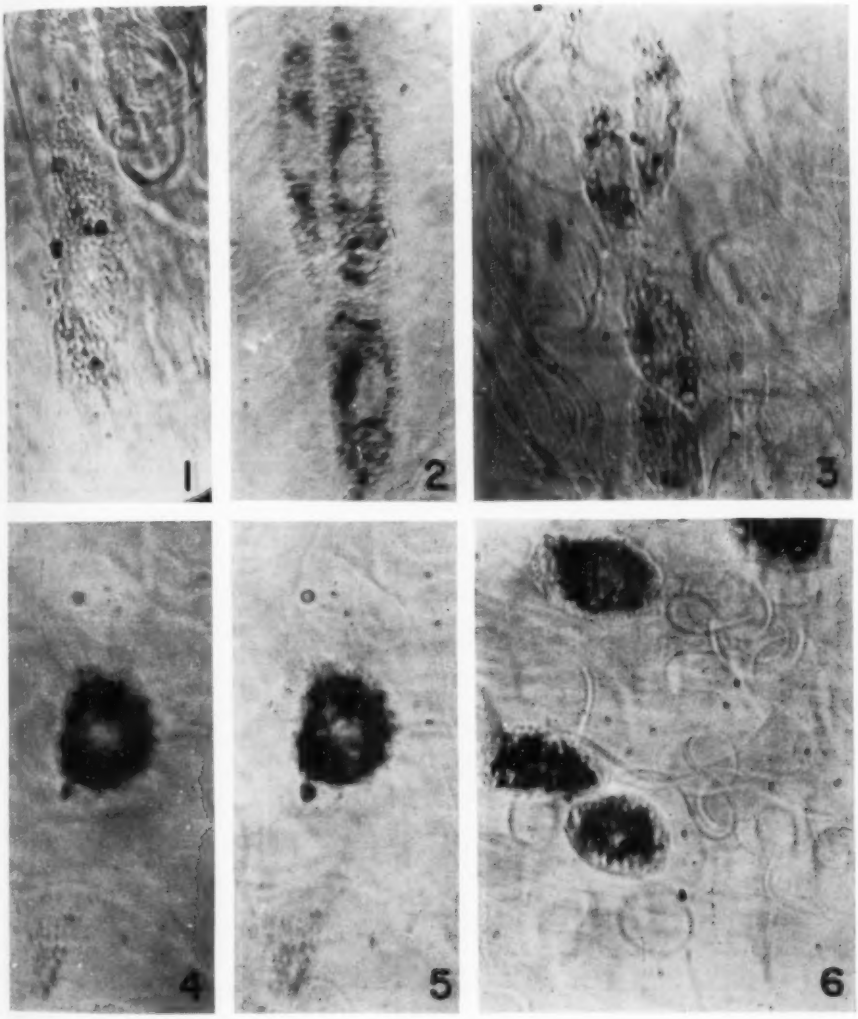
FIG. 10. Mouse no. 67. Film moistened with Locke's solution from which potassium chloride was omitted, but to which albumin (6.0%) was added. The "resting-wandering cells" were normally spread, and their cytoplasmic expansions contained abundant "fluid vacuoles". These tended to be of greater size than in films moistened with serum.

FIG. 11. Same film as for Figure 10. A group of "resting-wandering cells". The cells were more expanded than when films are moistened with serum, and the "fluid vacuoles" in the cytoplasmic expansions were larger than normal.

FIG. 12. Mouse no. 62. Film moistened with Locke's solution diluted with homologous serum (1:1). The "resting-wandering cells" did not contract. They remained normally expanded and with as plentiful "fluid vacuolization" as when moistened with serum alone. The "fluid vacuoles" show best at the lower right of the cell.



The tracings outline the extent of the cells as seen in the photographic reproductions on the page opposite.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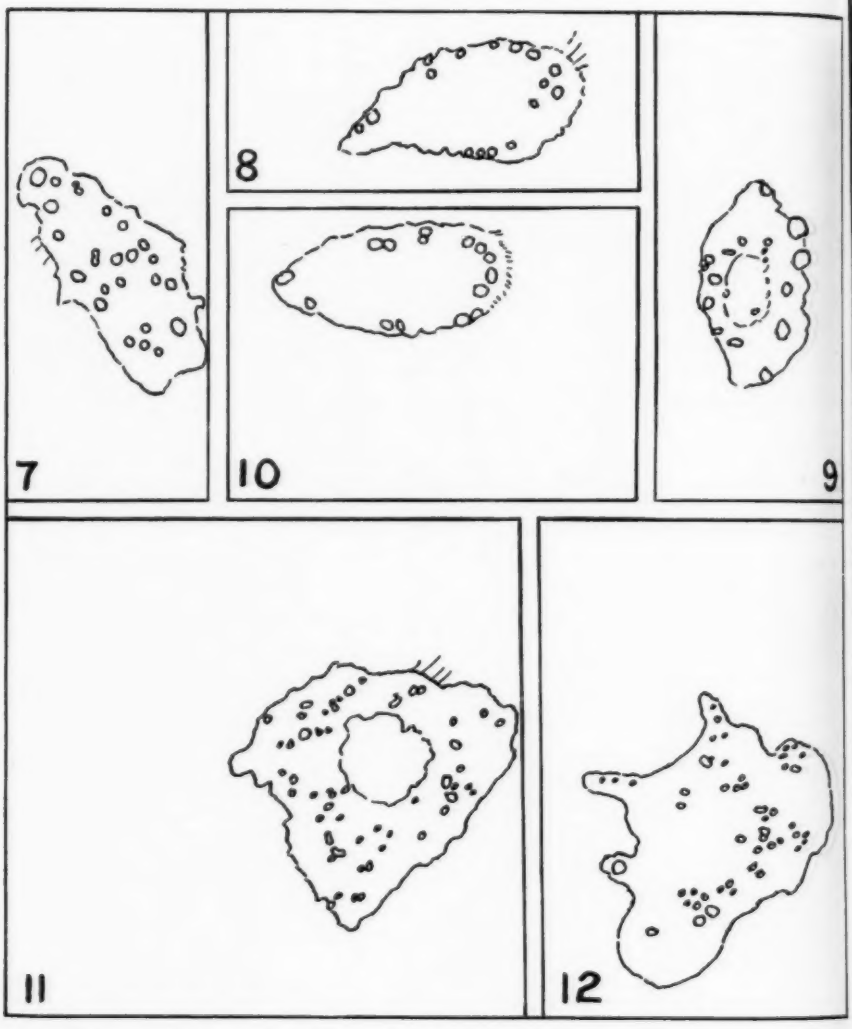

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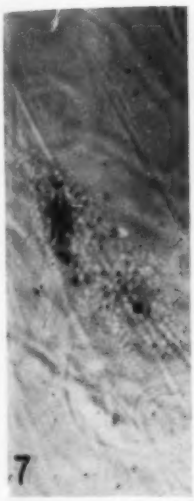
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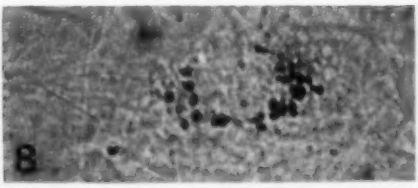
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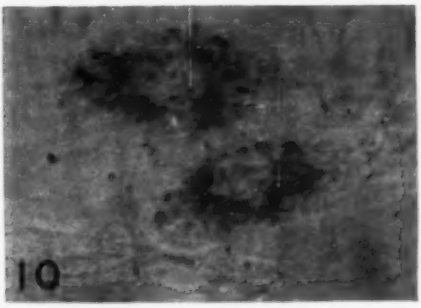
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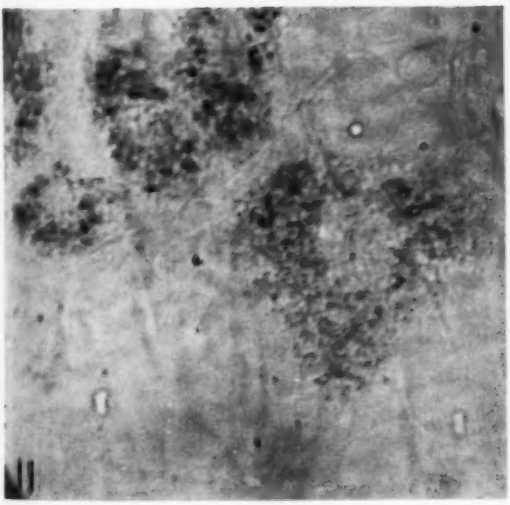
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THE LIVER IN SICKLE CELL ANEMIA

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Since the original description of sickle cell anemia by Herrick in 1910, surprisingly little attention has been given to the state of the liver by students of this disease. Several authors have reported the incidence of palpable enlargement of the liver (1, 2, 3). Others have noted that during crises transient hepatic enlargement occurred and the direct-reacting bilirubin increased to a considerable degree (3, 4). Scant data regarding liver function have been reported (5, 6).

CLINICAL STUDIES

Methods

Laboratory procedures were those routinely employed in the Johns Hopkins Hospital. The hematocrit was determined by the method of Wintrobe. Serum bilirubin was measured by the Van den Bergh technique. The foam test was employed for the detection of bile in the urine, and the concentration of urobilinogen was estimated by the method of Wallace and Diamond. In the bromsulphalein test, the concentration of dye in serum 30 minutes after intravenous injection of 5 mg. per kilogram of body weight was determined. The serum alkaline phosphatase activity was estimated by the method of Bodansky. Prothrombin time was determined by a one-stage method in which standardized thromboplastin was employed. The concentration of cholesterol in serum was measured by Bloor's method as modified by Buell. The serum protein concentration was determined by the Kjeldahl macro technique, and the albumin and globulin fractions estimated by the Kingsley modification of the Howe method. The cephalin flocculation test was performed according to the method of Hanger. The Shank and Hoagland modification of the thymol turbidity test was employed.

Of a large group of patients with sickle cell disease seen at the Johns Hopkins Hospital, fifty have been carefully studied with reference to hepatic function. Forty-eight of these fifty patients have been seen on repeated occasions, during crises and in asymptomatic intervals. Two patients have been under observation for 25 years, and several others have been seen repeatedly during periods in excess of 10 years. The status of the liver in many of these patients has been evaluated both during crisis and at other times. Forty-five of the patients were examined by the authors, while data concerning the remaining five were obtained solely from the hospital records. Thirty-nine patients had typical sickle-cell anemia, with persistent anemia, unremitting jaundice and recurrent episodes of pain in the abdomen and extremities. Eleven cases were classified as instances of "atypical sickle cell disease". All of the patients in this smaller group had at times anemia, jaundice, and with one exception symptoms

characteristic of sickle cell crisis.* However, these patients displayed features not usually encountered in sickle cell anemia. Five of the patients appeared to be heterozygous for the sickling character as determined by studies of their families. Careful and repeated tests, using several reducing agents to enhance sickling, showed that sickle cells were not demonstrable in the mother of three patients. Some of the children of two female patients did not display the sickling phenomenon. Eight of the eleven patients at times had no anemia and appeared to be remarkably well. Two are surviving at ages fifty-three and sixty-three. None of the eleven patients had leg ulcers, although such ulcers were present in fourteen of the thirty-nine typical cases. Nevertheless, nine patients in the atypical group have been observed to have severe crises with pain and fever, associated with evidences of increased red cell destruction. Four of these patients died between the ages of twenty-three and twenty-seven. No patients having only the sickle cell trait were included in this series. However, five individuals with the trait alone were studied using the same group of tests, and no evidence of hepatic dysfunction was found.

Size of the liver. The liver was palpable in 33 of 50 patients at some time during the period of observation. In most instances the degree of hepatic enlargement was moderate, the edge of the liver extending 5 cm. or less below the costal margin. In a few cases the liver extended below the umbilicus or even to the iliac crest. Palpable enlargement of the liver was the rule in patients in the first decade. In the second and third decades the liver was often not demonstrably enlarged, even in those patients observed to have had hepatic enlargement in childhood. Nevertheless, the most marked hepatic enlargement was encountered in patients in the third and fourth decades and was usually associated with evidence of impaired hepatic function. Often the liver was not palpable in asymptomatic periods, but became definitely enlarged during episodes of crisis.

Although cholelithiasis was common in this group of patients, in no case was hepatic enlargement attributable to obstruction of the common bile duct. Forty-six of the patients had cardiac enlargement, demonstrated by roentgenographic examination, and all of these had impressive cardiac murmurs often simulating those of rheumatic heart disease. However, congestive failure was a rare occurrence. The degree of hepatic enlargement was not related to the extent of cardiac involvement and except in three cases was not associated with peripheral edema or elevation of the venous pressure. Blood transfusions were administered very infrequently to most of these patients and in only one instance was there evidence of the occurrence of homologous serum hepatitis.

* The term "crisis" in this report is used to designate the characteristic episodes of pain and fever which occurred in patients with sickle cell disease, whether or not there was evidence of coincident increase in the rate of blood destruction.

Probably because of the small number of transfusions employed, hemachromatosis was not observed in these cases. Progressive enlargement of the liver with increasing age was often noted during adult life, but there was no correlation between the degree of hepatic enlargement and the severity of anemia. Characteristically the liver was smooth, of normal consistency and non-tender.

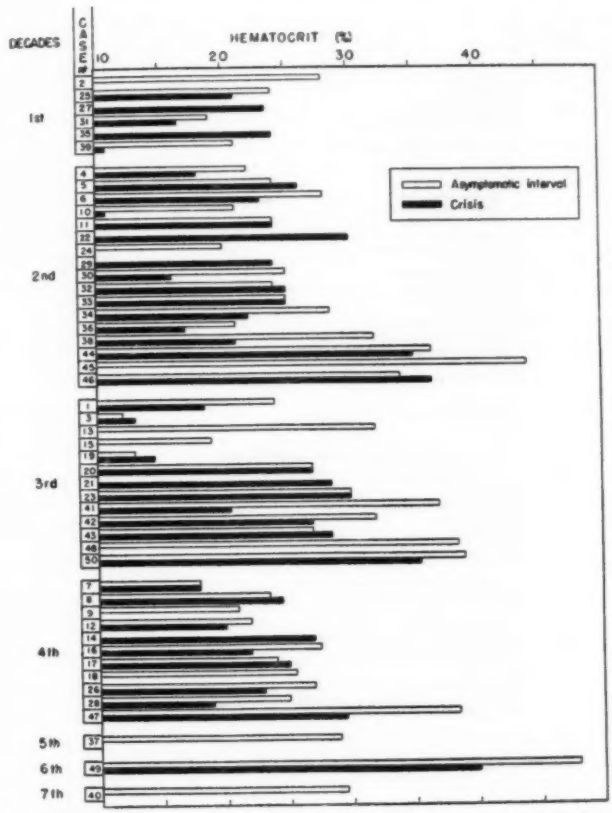

CHART 1. Hematocrit values of patients with sickle cell anemia. In this and in subsequent figures, a mean value is recorded when more than one determination was made.

In patients with atypical sickle cell disease hepatic enlargement was uncommon, even in patients in the older age groups.

Degree of jaundice. Jaundice was invariably present in all of the anemic patients in this series. In three patients with atypical sickle cell disease, jaundice was usually too slight to be detected clinically, but the serum bilirubin was at most times elevated above the normal level. The relatively high pro-

portion of indirect-reacting bilirubin and the absence of bile from the urine in most of the cases suggests that the jaundice for the most part was attributable to increased red cell destruction. There was considerable variation in the concentration of bilirubin in the serum of individual patients at different times as well as among different patients. The usual value was about 3 mg. per 100 ml.

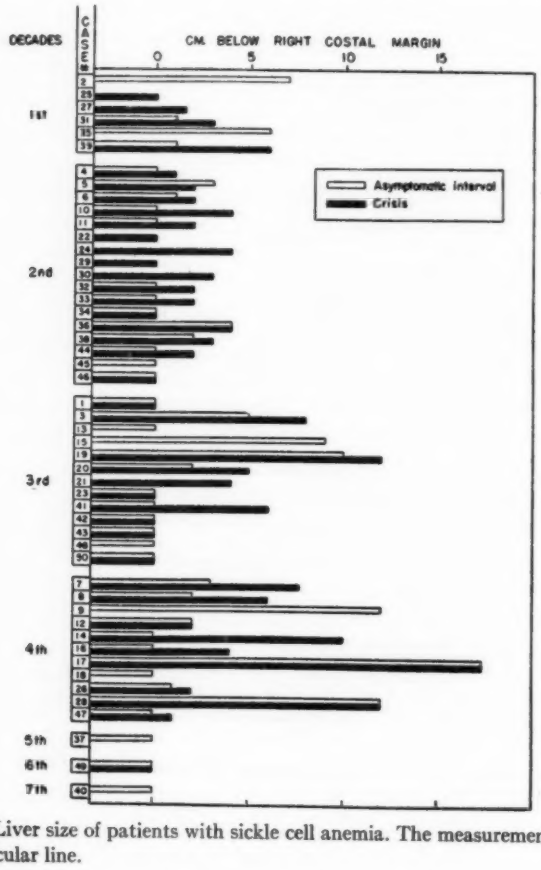

CHART 2. Liver size of patients with sickle cell anemia. The measurements were made in the mid-clavicular line.

The serum bilirubin often rose during episodes of crisis; however, a number of patients were observed to have values no higher during crisis than in asymptomatic intervals. In some patients very marked elevation of the serum bilirubin occurred on occasion, usually during a period of crisis. In these cases there was always a significantly high proportion of direct reacting bilirubin, together with bilirubinuria and marked evidence of hepatic dysfunction. The strikingly

high serum bilirubin values in these patients were associated with marked hepatic enlargement and could not be attributed to increased blood destruction, to obstruction of the common duct or to homologous serum hepatitis.

Cholecystitis and cholelithiasis. Only two patients in this group developed symptoms of gall bladder disease. The gall bladder was removed from one of these patients and was found to contain stones, and stones were demonstrated by cholecystogram in the other. A cholecystectomy had been performed on one patient at another hospital before the diagnosis of sickle cell anemia had been established. One other patient was subjected to cholecystectomy at a time when she displayed marked evidence of hepatic dysfunction. Although the gall bladder was filled with small pigment stones, the common duct was not obstructed and there was no evidence that the hepatic disorder was caused by the gall bladder disease.

Cholecystograms were performed on eighteen patients. In ten the roentgenograms were interpreted as normal, in three the gall bladder was not satisfactorily visualized and in five stones were demonstrated. All of the patients with abnormal cholecystograms were twenty-two years old or older, while five of the ten patients with normal gall bladder roentgenograms were twenty or younger. One patient whose cholecystogram appeared normal was demonstrated to have cholelithiasis at autopsy nine years later. Common duct obstruction was not recognized in any patient in this series, and no patient developed acute inflammatory disease of the gall bladder.

Bilirubinuria and urobilinogenuria. Six of the fifty patients were observed to have bile in the urine, four during crisis and two at other times. All of these six patients displayed evidence of severe liver disease. Three were demonstrated to have cirrhosis of the liver at autopsy and one other died of hepatic failure.

The concentration of urobilinogen in the urine, as estimated by the Wallace and Diamond method, was often increased in patients with sickle cell disease, both during and between crises. In patients with marked evidence of hepatic dysfunction, urobilinogen was present in the urine in high concentration. In those patients with marked jaundice, bilirubinuria and elevated serum alkaline phosphatase values, the urobilinogen concentration in the urine was always high, suggesting that the jaundice was not caused by common duct obstruction.

Bromsulphalein excretion. The rate of excretion of bromsulphalein was measured in thirty-seven cases. In nine the test was performed during crisis, and in four of these was also carried out during an asymptomatic interval. Normal excretion of bromsulphalein was usually observed during the first two decades, but in older individuals it was more common to find retention of the dye. In three of four patients examined both during crisis and at another time, there was no significant effect of crisis on bromsulphalein retention. Bromsulphalein tests were not performed on most of the patients who had very

high direct-reacting serum bilirubin concentrations during crisis. Presumably in these patients bromsulphalein retention would have been more marked. Patients with abnormal bromsulphalein excretion always displayed abnormalities of other liver function tests.

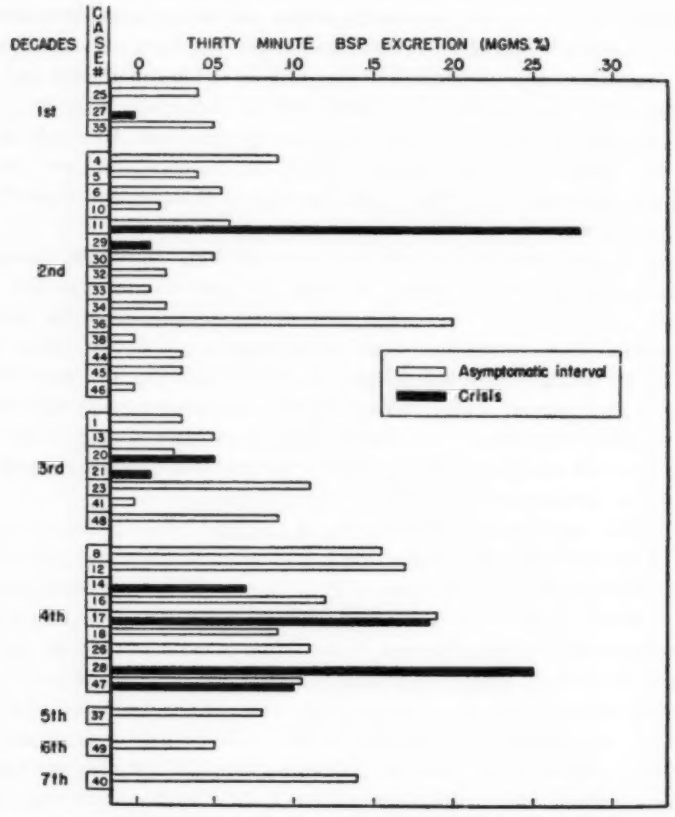

CHART 3. Bromsulphalein excretion by patients with sickle cell anemia. The concentration of the dye in serum 30 minutes after intravenous injection of 5 mg. per kilogram of body weight is recorded.

Serum alkaline phosphatase activity. The alkaline phosphatase activity of the serum was determined in forty-three patients, of whom fifteen were age fifteen or younger. In fourteen cases, determinations were made both in and out of crisis. In children the values in most instances did not exceed the normal for this age group. However, one child during crisis developed a striking rise of the alkaline phosphatase to 40 units at a time when there was a high direct-

reacting serum bilirubin in the absence of other evidence of obstruction of the biliary tract. In most of the other patients occurrence of crisis had no significant effect on phosphatase values. Eighteen of twenty-eight patients sixteen years of age or older had alkaline phosphatase values above 5 units, and in eight the values exceeded 10 units. None of these patients showed evidence of obstructive jaundice and the urinary excretion of urobilinogen was increased at the time

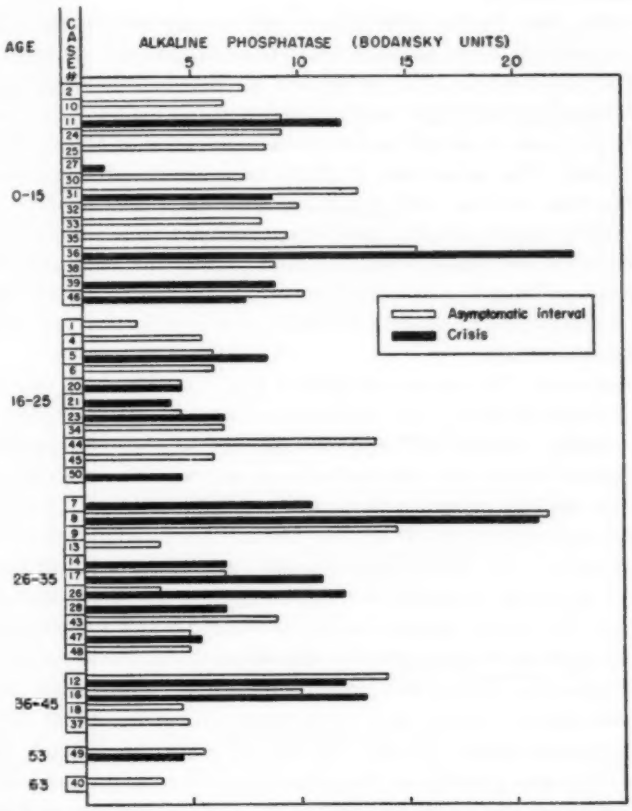

CHART 4. Alkaline phosphatase activity of the serum of patients with sickle cell anemia.

that the phosphatase was high. Cholecystectomy was performed on one patient when the phosphatase was elevated to 20 units. No evidence of biliary obstruction was found and the phosphatase remained persistently elevated for a period of observation of two years following the operation. Skeletal roentgenograms showed few abnormalities in the adult patients with high phosphatase values. The serum calcium, determined in ten cases, was normal except in two

patients in uremia who had slightly low values. The serum phosphorus was elevated in the two uremic patients but was normal in all other patients whose blood was examined for phosphatase activity. High phosphatase activity in this series of patients was always associated with other evidence of hepatic dysfunction. For these reasons it seems unlikely that the increased phosphatase activity could be attributed to alterations in the bones associated with increased marrow activity.

Prothrombin time. Prothrombin times were determined on the plasma of thirty-seven patients. Eight patients had determinations both during crisis and in asymptomatic intervals. The occurrence of crisis had no significant effect on the prothrombin time. Eight patients had prothrombin times in excess of 24 seconds (50 per cent of normal) and five others in excess of 22 seconds (65 per cent of normal). The occurrence of slight to moderate prolongation of the prothrombin time was not well correlated with other abnormalities of liver function, and in some patients little other evidence of hepatic disease was present. However, two patients with the most pronounced hepatic dysfunction had markedly prolonged prothrombin times. One of these patients displayed evidences of a hemorrhagic disorder when the prothrombin time was 53 seconds (20 per cent of normal).

Serum cholesterol. The serum cholesterol was determined on one or more occasions in fifteen patients. The values were well within normal limits in most instances, ranging between 125 and 240 mg. per 100 ml. One patient with manifestations of severe liver disease had a cholesterol value of 78 mg. per 100 ml. However, another patient who died of hepatic insufficiency had a serum cholesterol concentration of 380 mg. per 100 ml. immediately prior to death.

Serum proteins. The serum albumin and globulin concentrations were measured in forty-nine patients, in nineteen both in asymptomatic intervals and in crises. The serum albumin ranged between 5.8 and 2.4 grams per 100 ml., and ten patients at times had concentrations of 3.5 grams per 100 ml. or less. In four cases the albumin was 1 gram or more lower during crisis than in an asymptomatic period, but in other cases there was no significant difference. The serum globulin ranged between 2.0 and 4.7 grams per 100 ml. Twenty-five of the forty-nine patients at times had globulin concentrations of 3.0 or greater, twenty-one had concentrations of 3.5 or more, and ten patients had globulin concentrations of 4.0 grams per 100 ml. or greater. In two cases the serum globulin was considerably increased in crisis as compared to an asymptomatic interval.

Cephalin flocculation and thymol turbidity tests. Cephalin flocculation tests were performed one or more times on forty-four patients. A result of one-plus or greater was obtained in twenty-five patients, two-plus or greater in twenty-

three patients, and three-plus in fourteen patients. (In this laboratory one-plus is a significant positive result, and three-plus is the highest value reported). Fifteen patients had tests performed both during crisis and at other times and in these cases there was no apparent effect of crisis on the results of cephalin flocculation tests.

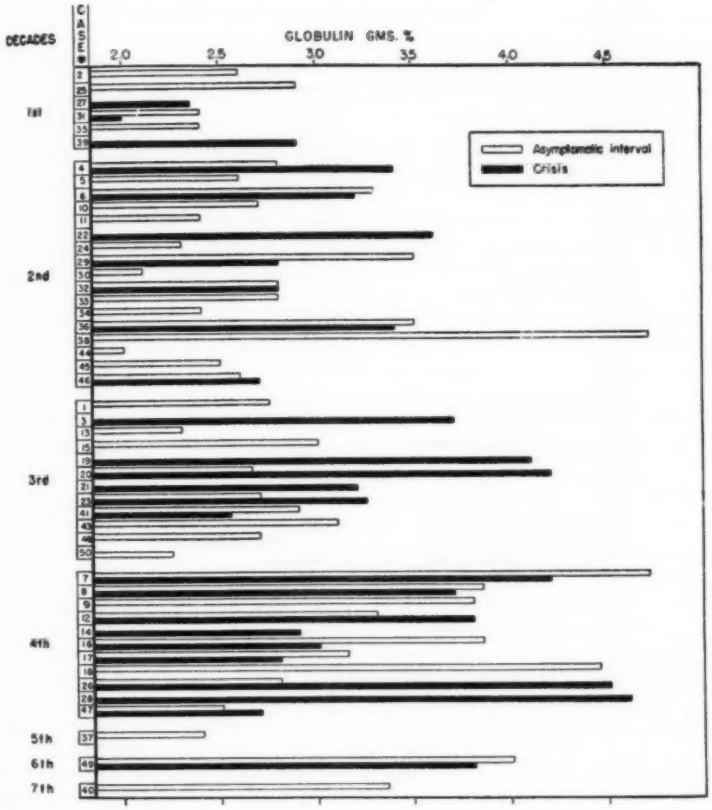

CHART 5. Serum globulin concentration (as determined by the Howe method) in patients with sickle cell anemia.

Thymol turbidity tests were performed on forty-one patients. The range of results was from 0.8 to 18.0 units. Values of 5 units or more were obtained in twenty cases and ten patients had values of 10 units or more. There was no significant effect of crisis on the results of thymol turbidity tests in fourteen patients examined in both phases of the disease.

In general, the most marked abnormalities in the results of these tests occurred in the older patients and were usually associated with other abnormalities of hepatic function.

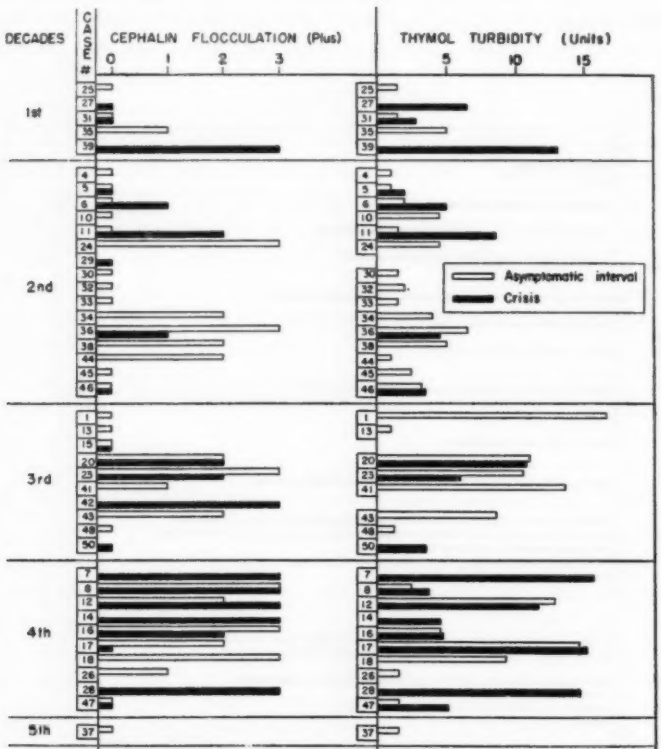

CHART 6. Cephalin flocculation and thymol turbidity tests on patients with sickle cell anemia. Three-plus is the maximum positive result of the cephalin flocculation test. A value in excess of 5 units is considered to be abnormal for the thymol turbidity test.

Illustrative Case Histories

The histories of four patients who showed pronounced evidence of hepatic dysfunction are summarized briefly.

Case 7. E. E. (J. H. H. #147393), a 33 year old colored male, was admitted to the hospital because of severe abdominal pain. There had been moderate intake of alcohol, averaging one pint per week for many years. The dietary intake had apparently been adequate. He had had chronic leg ulcers for 14 years and intermittent episodes of pain in the abdomen

and extremities since childhood. Two blood transfusions had been administered 10 years before admission because of anemia. Three weeks before admission he developed severe generalized abdominal pain associated with progressively increasing jaundice. One week later pain appeared also in the arms and legs and at this time he began to be troubled by pruritis. In the 4 days preceding admission he vomited blood-streaked material and passed several tarry stools. On the day before admission he began to have profuse bleeding from the nose, mouth and gums. The rectal temperature was 98.2°F, pulse 80, respirations 17 and blood pressure 118/70. He was an acutely ill, pale, stuporous young colored man with the typical habitus of sickle cell anemia. The skin, mucous membranes and sclerae were intensely jaundiced. Blood was oozing from the gums. The heart was enlarged and there was an apical systolic murmur. The liver extended 8 cm. below the costal margin and was smooth, firm and non-tender. Red cell count was 1.97 million, hemoglobin 7 grams, hematocrit 18 per cent, and white cell count 26,500 with 88 per cent neutrophils. The urine contained a trace of albumin and gave a four plus reaction for bile. The test for urobilinogen was positive in urine diluted 1:320. Blood non-protein nitrogen was 87 mg. and sugar 96 mg. per 100 ml. Serum bilirubin was 112 mg. per 100 ml. with 60 mg. direct reacting. Cephalin flocculation was three plus, thymol turbidity 15.5 units, alkaline phosphatase 10.4 Bodansky units, prothrombin time 53 seconds (20 per cent of normal) and serum cholesterol 380 mg. per 100 ml. Coma progressively deepened and the patient died on the second hospital day.

An autopsy was performed (#22118). The body was that of an icteric colored male with scars in the skin of the legs. The peritoneal cavity contained 250 ml. of clear yellow fluid and very small amounts of fluid were present in the pleural and pericardial cavities. The heart was dilated but not hypertrophied. An enlarged liver with smooth capsule and of rubbery consistency weighed 1940 grams. On cross section it presented a dark red, slightly rusty color with indistinct lobules. After formalin fixation the parenchyma became green. The gall bladder and bile ducts were normal and without stones. The spleen weighed 3 grams and consisted of rust-stained scar tissue. Swollen, smooth kidneys with an occasional cortical scar weighed 200 grams each and turned green with fixation. The bone marrow was everywhere hyperplastic and the cortices of ribs and skull seemed thin. The testes were small. On microscopic examination the regular pattern of the liver cords seemed to be distorted by extraordinary engorgement of the sinusoids by red cells, many of which were sickled. Most of these red cells were found within enormously enlarged Kupffer cells. Such cells, fairly bursting with phagocytized red cells, seemed to form plugs filling the sinusoids. Foci were seen in which isolated liver cells or small groups of liver cells were lost. In these areas the reticulum fibers were thickened and collapsed upon one another. Such foci were not all central in location, though most were near the central veins. Dilated bile canaliculi plugged with bile were frequently seen but small bile ducts were normal. Iron-positive pigment was present in both liver and Kupffer cells. The glomerular capillaries were engorged and the renal tubules showed prominent bile casts, with hemosiderin pigment in the cytoplasm of the tubule cells.

Case 8. G. E. (J. H. H. #151230), a 30 year old colored female was admitted to the hospital because of severe pain in the abdomen and extremities of several days' duration. She had had typical symptoms of sickle cell anemia since childhood and the diagnosis had been established 11 years before admission. There was no history of alcoholism or of hepatitis and dietary intake was fairly good. She had received several transfusions over a period of 10 years. On admission the rectal temperature was 103°F, pulse 100, respirations 24 and blood pressure 118/60. She appeared acutely ill with moderate pallor and intense icterus. The heart was enlarged with apical systolic and diastolic murmurs. The edge of the liver was

palable at the level of the umbilicus and was sharp, smooth and firm. The red cell count was 2.43 million, hemoglobin 8.4 grams, hematocrit 24.5 and white cell count 11,580 with 81 per cent neutrophils. The urine contained one-plus albumin and gave a positive reaction for bile. Urine diluted 1:320 gave a positive reaction for urobilinogen. The blood non-protein nitrogen was 33 and sugar 96 mg. per 100 ml. Serum bilirubin was 14 mg. per 100 ml. with 9 mg. direct reacting. Serum albumin was 3.2 grams and globulin 4.2 grams per 100 ml. Cephalin flocculation was three-plus, thymol turbidity 3.2 units, alkaline phosphatase 19.1 Bodansky units and prothrombin time 38 seconds (30 per cent of normal). A cholecystogram demonstrated the presence of many gall stones. Cholecystectomy was performed and at operation no evidence of common duct obstruction could be found. The patient was seen repeatedly in crisis and in asymptomatic intervals during a period of more than two years following the operation. The alkaline phosphatase remained persistently elevated, averaging 20 Bodansky units. The liver did not decrease in size and liver function tests continued to be abnormal. She had episodes of pulmonary edema, thought to be a manifestation of cardiac failure, and it was believed that she had rheumatic disease of the mitral valve. She died in what was evidently severe crisis. Just prior to death, liver function tests gave results very similar to those previously recorded.

At post-mortem examination (#23948) the body was found to be remarkably under developed and apparently poorly nourished. Icterus was obvious. The left pleural cavity was obliterated by fibrous adhesions. On the right, fibrous adhesions had not prevented the effusion of 400 ml. of dark yellow fluid. An edematous shaggy fibrinous pericarditis was present. The heart weighed only 320 grams in spite of this thick epicardium. Mitral valve leaflet scarring and fusion resulted in a mitral orifice of 0.5 cm. diameter. The left atrium and right ventricle were not hypertrophied. The lungs were heavy, moist and red-brown on cross section. A 2100 gram liver seemed enormously enlarged for the size of the body. It was grossly nodular with many broad grey scars. On cross section the color was red-brown with yellow foci. The gall bladder was missing with many adhesions in this area but the external bile ducts were normal. No stones were found in the common or hepatic ducts. A seven gram spleen was scarred and rusty. The kidneys weighed 200 and 220 grams. Superficial scars were present in the cortical surfaces. On cut surfaces, glomeruli were prominently seen as tiny red spots. No softening of the bones was noted but the marrow, including that of the femur, was dark red. Microscopic study of the liver showed a diffuse severe cirrhosis. The scars tended to be more severe in central areas but also involved portal or mid-zonal regions. Some were thin and fibrous. When involving periportal areas, bile duct proliferation was trivial. Many were peculiar broad bands of eosinophilic fibrillary material which extended between liver cords and cells and which had formed between such cells and the sinusoidal lumens. Here the liver cells were extremely atrophic. Rarely, broader hyaline sheets of scar were essentially acellular. Erythrophagocytosis was seen in some areas. Many cells, thought to be Kupffer cells, were distended with small droplets of lipoid giving a foamy cytoplasm in the routine section. These lay singly or in small clusters in sinusoids and in the latter instances, displaced the liver cells. Accumulations of leucocytes which formed small nodules in the liver lobule were seen as well as larger areas of the loss of liver cells in which many leucocytes remained. Such foci did not show a strict central distribution. No fresh liver cell necroses were seen. Dilated bile canaliculi were not numerous. Extramedullary hematopoesis was observed. Sickled red cells were plainly visible in sinusoids and in the Kupffer cells. Liver cells often contained small sudanophilic droplets.

Case 28. (J. H. H. #280018), a 30 year old colored male was admitted to the hospital because of severe pain in the abdomen, back and legs for 36 hours. The diagnosis of sickle cell

anemia had been established 18 years before, and he had been seen on many occasions both during crises and at other times. The liver had been persistently enlarged, measuring 8 to 12 cm. below the costal margin. There was no history of alcoholism or of hepatitis and the diet was considered to be adequate. He had had several transfusions, but none in the 10 years prior to admission. For two years prior to admission he had noted increasing dyspnea and orthopnea. Deepening jaundice and dark urine accompanied the episode which led to his admission. The rectal temperature was 99.2°F, pulse 132, respirations 40 and blood pressure 110/60. He was an acutely ill, deeply jaundiced, comatose young man displaying the usual habitus of sickle cell anemia. The heart was enlarged and loud systolic murmurs were heard over the mitral and pulmonic areas. The liver was firm, smooth and non-tender and extended to the iliac crest. The red cell count was 2.1 million, hemoglobin 6.0 grams, hematocrit 19 and white cell count 38,000 with 4 per cent myelocytes, 20 per cent juveniles and 45 per cent neutrophils. No urine was obtained. The blood non-protein nitrogen was 73 and sugar 23 mg. per 100 ml. Serum bilirubin was 20 mg. per 100 ml. with 14.8 mg. direct reacting. Cephalin flocculation was three plus, thymol turbidity 14.3 units and alkaline phosphatase 6.6 Bodansky units. He died on the day of admission.

An autopsy was performed (#22317). The body was that of a thin, poorly muscled, jaundiced colored male. Scars were present in the skin of the lower legs. The left pleural cavity contained 600 ml. of dark yellow fluid. The heart was dilated and the right ventricle was hypertrophied, its myocardium measuring 10 mm. in thickness. The liver was coarsely nodular, firm and rubbery and weighed 2600 grams. On the cut surface nodules were seen varying in size from 1 to 5 mm. separated by bile-stained fibrous tissue. Some of these scars were broad. No gall stones were seen and the external biliary tree was normal. A chronic peptic ulcer was present in the stomach. The kidneys were finely granular and weighed 200 grams each. The cut surfaces showed atrophied cortices marked by many red petechiae. The testes were small. On microscopic examination the liver contained some very broad scars in which prominent bile duct proliferation had occurred typical of healed necroses. In addition, innumerable fine scars criss-crossed through the liver dividing it into regenerating nodules of varying size. These scars did not seem to arise from central zones. In the central portions of some nodules fresh necroses were present as well as other small foci marked by loss of liver cells and accumulation of pigment-laden macrophages. Packed masses of sickled red cells were seen within Kupffer cells and in the dilated sinusoids. Heavy deposits of hemosiderin were seen in the liver and Kupffer cells but the scars were relatively free of such pigment. The spleen was replaced by iron-encrusted scar. The pulmonary vessels were engorged and contained an occasional organized and recanalized thrombus associated with intimal arteriosclerosis. Renal glomerular capillaries were engorged with sickled red cells and an occasional scarred or even freshly necrotic tuft was found. Much iron pigment was present in the tubule cells.

Case 36. R. W. (J. H. H. #A40069), a 10 year old colored male was admitted to the hospital because of weakness and severe pain in the back, chest and knees of 12 hours duration. He had been observed at this hospital on frequent occasions since the age of 5 when the diagnosis of sickle cell anemia had been established. He had had many severe crises. The oral temperature was 98.6°F, pulse 90, respirations 30 and blood pressure 100/64. He was an acutely ill boy with moderate pallor and jaundice. The heart was enlarged and a soft systolic murmur was audible. The liver was palpable 4 cm. below the costal margin and was slightly tender. The red cell count was 2.48 million, hemoglobin 8.0 grams, hematocrit 24 per cent and white cell count 28,800 with 5 per cent juveniles and 85 per cent neutrophils. Blood non-protein nitrogen was 42 and sugar 100 mg. per 100 ml. Serum bilirubin was 10.0

mg. per 100 ml. with 4.0 mg. direct reacting. Cephalin flocculation was one plus, thymol turbidity 2.6 units and alkaline phosphatase 40.4 Bodansky units. Serum calcium was 11.2 mg., phosphorus 2.6 mg., albumin 4.6 grams and globulin 3.5 grams per 100 ml. His pain gradually subsided over a period of 5 days, and the liver decreased in size. After 3 weeks, the liver edge was felt at the costal margin. The alkaline phosphatase decreased to 13.4 units in one week and to 5.4 units in 3 weeks. Within a week the serum bilirubin had dropped to 1.5 mg. He has subsequently been examined repeatedly both during crisis and in asymptomatic intervals. He has displayed considerable evidence of abnormal hepatic function at these times (see Table I).

PATHOLOGICAL STUDIES

In attempting to understand the pathogenesis of the clinical manifestations of liver dysfunction which have been discussed, a review of the morphological changes which have been observed in the livers of patients dying with sickle cell disease is essential. For this reason, twenty-one autopsied cases of patients dying with clinical evidence of severe sickle cell disease have been studied from the files of the Department of Pathology for the years 1920 to 1951. Although the autopsied cases are not identical with those studied clinically in this report, such a review is pertinent. Cases No. 3, 6, 8, 19, 28, 42 and 43 from the clinical section were included in this post-mortem study and will be discussed in particular detail.

The ages of these patients ranged from 14 months to 38 years. There were 8 patients 7 years old or younger and 13 were 15 years or older. Grossly, the liver was enlarged in 14 cases and normal or small in the remaining 7. Of the 13 patients over 15 years of age, 10 had livers increased in size, while 4 of the 8 cases under 15 showed enlarged livers. The liver capsules were smooth except in 4 cases where obvious nodular cirrhosis was present. The cut surfaces of the non-cirrhotic livers were sometimes pale, even yellow in color with accentuation of the markings by prominent yellow or sometimes dark red central zones. Some livers were described as having a deep red color and one pathologist thought that a liver resembled the spleen seen in cases of congenital hemolytic icterus. In the protocols of others, a brown or bronze color of the cut surface suggested hemosiderosis to the prosectors. No abnormalities of the bile ducts or gall bladder were noted but gall stones were described in one case in which 3 stones were found lying in the common duct near the ampulla of a 29 year old patient. One patient had had the gall bladder removed two years before death. At autopsy the common and hepatic ducts were normal and no stones were found.

Microscopically, the most prominent finding in almost all livers was a congestion of the sinusoids by sickled red cells and a widespread phagocytosis of large numbers of red cells by the Kupffer cells. The latter was absent in only two cases and was marked in many. The Kupffer cells were sometimes so distended with red cells as to make location of the cytoplasm or nuclei difficult

or impossible and the resulting lump of perhaps 10–20 red cells caused widening of liver sinusoids. In most livers, hemosiderin as well as other pigment granules were present both in Kupffer and in liver cells. In two cases, in addition to these changes, an occasional Kupffer cell was foamy.

In many cases the liver cells showed diffuse atrophy and this was most noticeable in central zones. Sometimes fine droplets of fat were present in the central cells. In those livers most severely congested and with the most prominent erythrophagocytosis, the architecture of the liver cords seemed irregular and rarely the loss of isolated hepatic cells was clearly apparent. In several, small clumps of leucocytes in gaps in the liver cords indicated more definitely the death of single liver cells. In five cases, more widespread necrosis of the liver parenchyma was present, with prominent fresh centralobular necroses in three cases and necroses in the central portions of liver-cell nodules in two of the cases of cirrhosis.

In six cases without cirrhosis there were tiny focal areas where liver cells had been lost. These tiny areas bore no constant relationship to either central or periportal regions. Some of these foci revealed early fibrosis while others resembled only the thickening and condensation of the reticular fibers of the stroma following the loss of liver cells. Pigment was often seen in macrophages in these lesions.

Four of the cases, one child of 6½ years and three adults, had advanced nodular cirrhosis. In these, there were both broad and fine scars completely disrupting the normal structure. These were not typically of the central or so called "cardiac" type of cirrhosis although in some areas central scarring was obvious. Some of the scars were large and prominent bile duct proliferation was seen embedded in the connective tissue. These lesions were quite characteristic of healed massive necrosis but in addition fine radiating scars penetrated all regions of the livers. Two of the cirrhotic livers showed trivial pigment deposits but one contained large amounts of iron-positive pigment in the parenchymal and in the Kupffer cells without pigmentation of the scar. The pancreas and lymph nodes of the latter case also contained small amounts of iron-positive pigment. This patient had received a total of five transfusions, the last of which had been administered fifteen years before death. One patient had the most peculiar cirrhosis with broad bands of loose fibrillary material lying between atrophic liver cells, with foci of healed necroses and with peculiar accumulations of foamy macrophages. This patient had mitral stenosis, and a history of cardiac decompensation but the cirrhosis was certainly not the usual variety of cardiac cirrhosis.

Dilated bile canaliculi were found in three cases. Two of these were associated with diffuse liver cell necroses but those seen in an adult's liver in the absence of cirrhosis or necroses had no recognizable cause. It should be pointed out that bile canaliculi dilated with bile plugs may be seen as a manifestation of poorly

TABLE I

Liver Function in Sickle Cell Anemia

Cases 40 through 50 comprise the group designated as "atypical sickle cell disease". When multiple determinations were made, the range of results is indicated by the two values given.

CASE	HISTORY NUMBER	PATIENT (AGE, SEX, RACE)	HEMATOCRIT		LIVER SIZE		SERUM BILIRUBIN TOTAL (DIRECT)		SERUM ALBUMIN		SERUM GLOBULIN		CEPHALIN FLOCCULATION (0 TO +++)		THYMOL TURBIDITY		ALKALINE PHOSPHATASE		BSP RETENTION		PROTHROMBIN TIME		UROBILINOGEN IN URINE (0 TO ++++)		BILE IN URINE (0 TO ++++)		GALL BLADDER X-RAY
			A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	
			%		cm.		mg./100 ml.		Gm./100 ml.		Gm./100 ml.				units		units		mg./100 ml.		sec.						
1	283991	C. B. 24 CF	24	17	0	0	2.5(1.2)	2.0(0.8)	4.4	—	2.6	—	0	—	16.6	—	2.7	—	0.3	—	22	—	+	+	0	0	—
				20					4.6		2.9																
2	A28924	G. B. 7 WM	28	—	5	—	1.7(0.5)	—	4.0	—	2.6	—	—	—	—	—	7.6	—	—	—	—	—	—	—	0	—	—
3	135000	H. B. 29 CF	12	13	5	8	1.6	5.4		2.6		3.7	—	—	—	—	—	—	—	—	—	—	—	+	+	0	neg.
4	342270	E. C. 19 CM	22	18	0	1	4.7(1.1)	3.5(1.4)	4.9	3.9	2.8	3.4	0	—	1.2	—	5.6	—	0.9	—	16	—	++++	—	0	0	neg.
															0.6						26						
5	445803	D. C. 17 CF	22	25	3	1	4.6(2.8)	6.6(1.2)	4.9	—	2.6	—	0	0	0.8	1.8	4.3	8.3	0.4	—	20	19	+	++++	0	0	neg.
			26	27		3									1.3		7.6										
6	169762	W. C. 19 CM	28	23	1	2	2.2(1.2)	4.0(1.1)	4.3	4.6	2.9	3.2	0	0	1.6	4.9	5.8	—	0.5	—	17	—	+	0	0	0	—
							3.2(1.2)		4.7		3.7		++		2.5		6.1		0.6		19		++++		0		
7	147393	E. E. 33 CM	16	18	3	8	1.7	112(60)	3.7	3.3	4.7	4.2	—	+++	—	15.5	—	10.4	—	—	—	53	+++	++++	0	++++	—
			20																								
8	151230	G. E. 31 CF	23	23	2	2	4.7(2.5)	3.7(2.3)	3.5	3.2	4.0	3.2	++	++	1.8	1.8	18.4	19.1	1.3	—	19	20	—	+++	—	0	pos.
			24	26		10	3.8(2.2)	14(9.0)	3.9	4.1	3.7	4.2	+++	+++	3.6	5.9	24.5	23.3	1.8		22	38		+++		++	
9	294049	J. E. 31 CM	19	—	12	—	6.2(2.6)	—	2.9	—	3.8	—	—	—	—	—	14.5	—	—	—	15	—	++++	—	—	—	not vis.
			23				8.8(4.5)		3.2		3.8																
10	235589	E. G. 12 CM	21	9	0	4	2.9(1.2)	—	5.1	—	2.7	—	0	—	4.5	—	6.7	—	0.3	—	17	—	+	—	0	—	—
																			0.4								
11	393459	E. G. 11 CF	24	24	0	2	2.7(1.2)	2.8(1.4)	4.8	—	2.5	—	0	++	1.2	8.5	9.0	12.0	0.6	2.8	24	—	+	—	0	0	—
									4.9		2.3		0		1.3		9.2										
12	575769	M. H. 38 CF	22	20	2	2	2.7(1.1)	2.9(1.2)	4.0	3.8	3.3	3.8	++	+++	12.8	11.5	14.0	11.8	1.7	—	17	21	+	+	0	0	—
13	420348	M. H. 26 CF	8*	—	0	—	1.6(0.8)	—	4.7	—	2.3	—	0	—	1.0	—	3.4	—	0.5	—	21	—	—	—	—	—	not vis.
			32																								
14	383997	P. H. 35 CF	20*	24	0	10	1.4(0.6)	1.6(0.8)	2.6*	4.1	2.9*	2.9	—	0	—	2.5	—	5.0	—	0.7	—	22	—	++++	0	0	neg.
				30				7.2(4.4)	3.3*		3.7*			+++		6.5		8.1						++++			
15	224804	W. H. 20 CM	19	—	9	—	3.0	—	3.9	—	3.0	—	0	0	—	—	—	—	—	—	—	—	++	—	0	—	—
16	557598	P. H. 38 CM	28	22	0	4	6.0(2.3)	9.0(3.7)	3.9	4.0	3.7	3.0	+++	++	3.0	3.8	8.0	9.8	1.2	—	16	—	++	—	+	+	—
			27				6.4(2.5)		4.2		4.0		+++		5.8	5.3	11.8	16.0			24						
17	181352	L. H. 33 CM	20	25	17	17	8.7(3.3)	11(5.6)	3.7	3.5	2.8	2.8	+	0	11.4	15.0	4.7	10.2	2.0	1.9	19	18	++	—	0	—	pos.
			28				43(22.0)		4.2		3.3		+++		17.6		8.2	12.1		2.0			+++		+		

17	181352	L. H. 33 CM	20	25	17	17	8.7(3.3)	11(5.6)	3.7	3.5	2.8	2.8	+	0	11.4	15.0	4.7	10.2	2.0	1.9	19	18	++	—	0	—	pos.
			28				43(22.0)		4.2		3.5		+++		17.6		8.2	12.1		2.0			+++		+		
18	195758	D. J. 38 CM	23	—	0	—	4.0(1.8)	—	4.1	—	4.2	—	+++	—	9.2	—	4.4	—	0.5	—	16	—	0	—	0	—	pos.
			28						4.3		4.7								1.3								
19	193262	J. J. 23 CM	13	7	10	12	—	1.6	—	2.4	—	4.1	—	—	—	—	—	—	—	—	—	—	+	—	0	0	—
				22				9.3															++				
20	247844	C. L. 25 CF	24	24	2	4	1.5(1.1)	2.5(0.9)	4.4	3.1	2.0	3.7	++	0	4.0	9.6	4.2	3.9	0.2	0.2	—	22	++++	+	0	0	pos.
			30	30		6	2.2(1.1)	4.1(0.9)	4.6	4.7	3.3	4.7	++	++	18.0	11.0	4.4	4.8	0.5	0.8	—	24		++			
21	182620	S. L. 23 CM	—	28	—	2	—	1.1	—	3.9	—	3.2	—	—	—	—	—	4.0	—	tr.	—	—	—	+	—	0	—
				29		6																					
22	384291	F. M. 18 CF	—	30	—	0	—	3.0(0.9)	—	3.9	—	3.6	—	—	—	—	—	—	—	—	—	—	—	++	—	0	neg.
23	446340	A. M. 22 CF	26	29	0	0	3.5(1.1)	3.7(1.4)	4.9	4.7	2.7	2.9	++	+	5.9	4.8	4.4	4.7	0.3	—	19	—	+	0	0	0	not vis.
			34	31			5.2(1.1)	8.7(1.5)		4.8		3.6	+++	++	14.8	7.0		8.3	1.9		23			++			
24	A19125	B. M. 11 CF	20	—	—	2	2.0(1.4)	—	4.7	—	2.3	—	+++	—	4.3	—	9.1	—	—	—	—	—	—	—	—	—	—
						6																					
25	A52945	R. M. 5 CM	24	21	—	0	2.2(1.5)	—	4.4	—	2.9	—	0	—	1.5	—	8.6	—	0.4	—	20	—	—	—	—	—	—
26	122176	J. N. 32 CF	25	18	0	0	4.0(1.2)	3.3	5.1	2.4	2.8	4.5	0	—	1.3	—	2.9	11.4	1.1	—	16	—	0	+++	0	0	pos.
			29	28	2	4		4.0					++		1.4		4.2	12.2					++	++++			
27	A46255	B. P. 6 CF	—	20	—	1	—	1.8(0.8)	—	4.9	—	2.3	—	0	—	6.4	—	1.0	—	0	—	—	0	0	0	0	—
				31		2				5.1		2.4															
28	280018	W. R. 30 CM	25	19	12	12	—	20(14.8)	—	4.4	—	4.6	—	+++	—	14.3	—	6.6	—	2.5	—	—	—	++	—	+	neg.
																								+++		++	
29	A23837	G. R. 10 CM	—	24	—	0	—	2.9	4.4	4.5	3.5	2.8	—	0	—	—	—	—	—	0.1	—	—	—	++++	—	0	—
30	A39976	J. R. 14 CM	24	14	—	3	2.2(1.5)	—	5.0	—	2.1	—	0	—	1.3	—	7.7	—	0.5	—	20	—	—	—	—	—	—
			26																								
31	A28620	R. R. 9 CM	19	14	0	2	2.3(1.1)	2.2(0.8)	5.3	5.3	2.4	2.0	0	0	1.3	2.8	12.8	8.8	—	—	25	—	++++	—	0	0	—
				19	2	4															25						
32	A19330	L. T. 11 CM	24	24	0	1	2.7(1.4)	—	4.7	3.6	2.8	2.8	0	—	1.8	—	10.0	—	0.2	—	25	—	+	+	0	0	—
				26		3																					
33	168696	D. T. 15 CM	25	25	0	1	1.3(0.8)	—	4.8	—	2.8	—	0	—	1.4	—	8.3	—	0.1	—	25	—	0	+++	0	0	—
						3																					
34	103460	R. W. 18 CF	26	22	0	0	1.8(0.8)	—	4.6	—	2.4	—	+	—	3.8	—	6.1	—	0.2	—	18	—	0	0	0	0	—
			31										++		4.3		7.0										
35	A61621	F. W. 8 CM	—	25	6	—	2.8(1.2)	—	5.0	—	2.4	—	0	—	3.6	—	9.2	—	0.5	—	24	—	—	—	—	—	—
							3.8(1.5)						++		6.4		9.4										
36	A40069	R. W. 12 CM	18	17	4	4	8.0(3.4)	5.7(1.2)	4.3	3.4	3.5	3.3	+++	0	6.4	2.6	15.4	5.2	2.0	—	22	21	—	—	—	0	neg.
			24					10.0(4.0)		5.8		3.5		+		6.0		40.4									
37	583191	G. S. 42 CF	29	—	0	—	2.8(1.1)	—	4.9	—	2.4	—	0	—	1.4	—	4.9	—	0.8	—	20	—	++++	—	0	—	—
38	591808	W. C. 14 CM	32	21	2	3	1.6(1.0)	—	4.0	—	4.7	—	++	—	4.8	—	8.8	—	0	—	24	—	++++	—	0	—	neg.
																					29						
39	A71693	A. D. 3 CM	21	5	1	6	—	4.6(2.0)	—	4.5	—	2.7	—	+++	—	13.0	—	8.8	—	—	—	16	—	—	—	—	—
				15																							
40	354466	J. C. 63 CM	29	—	0	—	1.0(0.8)	—	4.1	—	3.2	—	+	—	9.2	—	3.2	—	1.4	—	18	—	+	—	0	0	—
			30						4.3		3.5						3.6										

TABLE I—*Continued*

CASE	HISTORY NUMBER	PATIENT (AGE, RACE, SEX)	HEMATOCRIT		LIVER SIZE		SERUM BILIRUBIN TOTAL (DIRECT)		SERUM ALBUMIN		SERUM GLOBULIN		CEPHALIN FLOCCULATION (0 TO ++++)		THYMOL TURBIDITY		ALKALINE PHOSPHATASE		BSP RETENTION		PROTHROMBIN TIME		UROBILINOGEN IN URINE (0 TO ++++)		BILE IN URINE (0 TO ++++)		GALL BLADDER X-RAY
			A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	
			%		cm.		mg./100 ml.		Gm./100 ml.		Gm./100 ml.				units		units		mg./100 ml.		sec.						
41	305141	E. F. 23 CF	35	13	0	6	1.5(0.8)	1.6(0.7)	4.0	4.0	2.9	2.3	+	—	13.6	—	—	—	0	—	—	—	+	0	0	0	—
			39	28						4.1		2.8															
42	281428	H. H. 27 CF	29	27*	0	0	3.2(1.0)	—	—	—	—	—	—	+++	—	—	—	—	—	—	—	—	—	—	—	—	—
			32																								
43	127304	D. J. 26 CF	27	22	0	0	2.7(1.8)	—	3.6	—	3.1	—	++	—	5.1	—	8.8	—	—	—	—	—	—	+	0	0	—
				35			5.5(3.2)								11.8									++			
44	459508	E. N. 17 CM	32	35	0	2	2.2(1.0)	1.5(0.8)	4.8	—	2.0	—	++	—	0.8	—	13.7	—	0.3	—	21	—	0	+++	0	0	—
			41																								
45	397494	G. N. 19 CM	44	—	0	—	1.6(0.8)	—	5.0	—	2.5	—	0	—	2.3	—	5.8	—	0.3	—	22	—	—	—	0	—	—
46	503965	T. N. 14 CF	34	32	0	0	3.0(1.4)	4.6(1.2)	4.2	4.6	2.6	2.7	0	0	3.2	3.5	10.2	7.3	0	—	24	26	+	+++	0	0	—
				41																							
47	143970	J. N. 30 CM	36	29	0	0	3.2(1.2)	7.9(2.7)	5.0	4.0	2.5	2.7	0	0	1.6	4.9	3.8	4.8	0.5	1.0	16	20	++	0	0	0	neg.
			41	30	0	2	3.4(1.4)								1.8		4.8		1.6		20			+++			
48	276138	R. N. 27 CM	37	—	0	—	3.7(0.8)	—	5.0	—	2.7	—	0	—	1.2	—	4.6	—	0.9	—	19	—	+	—	0	0	—
			40																								
49	182730	S. S. 53 CM	45	35	0	0	1.8(0.8)	1.4(0.8)	4.6	4.0	4.0	3.8	0	+++	3.2	9.2	4.4	4.8	0.5	—	20	20	+	+	0	0	neg.
			51	45									++		5.9		6.2						+				
50	445265	P. Z. 24 WM	35	33	0	0	1.4(0.8)	1.4(0.8)	—	5.3	—	2.2	—	0	—	3.5	—	3.8	—	—	—	22	—	0	—	0	—
			43	38				5.2(2.5)		5.3		2.3					4.9							+++			

* Determination made during pregnancy.
A—Asymptomatic interval.
C—Crisis.

functioning hepatic cells, whatever the cause, in the absence of obstructive lesions of the bile ducts. An isolated and obscure finding was the presence of peculiar tiny hyaline fibrin-like thrombi in the sinusoids of two cases.

Among the cases studied in detail clinically there were seven which were included in the series studied pathologically. Clinical case # 3 with a palpable liver, a high direct bilirubin and a reversal of the A/G ratio showed at autopsy an enlarged liver, very prominent dilatation of sinusoids, and erythrophagocytosis. In addition, tiny foci of liver cell loss were seen. This patient had gall stones and a dilated common duct but no intra-hepatic evidence of biliary obstruction was noted. Case # 7 with severe functional impairment of the liver including a very high direct bilirubin showed an enlarged liver with extraordinary erythrophagocytosis, and focal loss of tiny groups of liver cells. The bile canaliculi occasionally were distended with bile plugs but no gall stones or other cause of mechanical obstruction were discovered. Case # 8 showed a peculiar and severe cirrhosis with prominent liver cell atrophy, healed focal necroses, accumulation of foamy macrophages and areas where Kupffer cell erythrophagocytosis was prominent. Dilated bile canaliculi were present. No stones were seen. The functional abnormalities of case # 19 were associated with cirrhosis of the liver as well as dilatation of sinusoids, erythrophagocytosis and recent focal necroses of liver cells. The severe functional changes in case # 28 are to be matched with prominent nodular cirrhosis, erythrophagocytosis and recent and fresh liver cell necroses.

Of the "atypical" cases of sickle cell disease, the positive cephalin flocculation in case # 42 can be compared only with liver cell atrophy, marked congestion of the sinusoids, and mild erythrophagocytosis. The mild functional changes found in case # 43 were associated with very prominent erythrophagocytosis with loss of isolated liver cells and rare focal scars. Both of these latter patients had non-palpable livers clinically, yet livers of 2300 and 2200 grams respectively were found at autopsy.

DISCUSSION

The scanty data available in the literature suggest that hepatic dysfunction may occur in patients with sickle cell anemia (4–7). Evidence of liver disease was frequently encountered in the patients described in this report. There are a number of mechanisms which might account for the development of the hepatic disorder. Prolonged hemolytic anemia with increased excretion of bilirubin predisposes to the formation of gall stones. For this reason one might expect to encounter a high incidence of clinical gall bladder disease and common duct obstruction in patients with sickle cell anemia. In congenital hemolytic jaundice, a disease somewhat comparable to sickle cell anemia, cholecystitis is not uncommon. It is surprising therefore to note that clinical manifestations of gall bladder disease are rare in sickle cell anemia, even though gall stones are

frequently present. Gall stones were demonstrated in a number of our living patients and the failure to find stones in more than one of the 21 autopsied cases is peculiar. Weems (8) found gall stones in 12 of 44 autopsies of patients with sickle cell disease. The clinical diagnosis of common duct obstruction was not established in any case in the present series and no instance has been reported in the literature (7). In the cases described in this report the aberrations of liver function tests frequently suggested the possibility of obstructive jaundice because of the high direct reacting bilirubin and elevated alkaline phosphatase. However, in only one case was stone in the common duct demonstrated at autopsy. It is clear that the hepatic disorder associated with sickle cell anemia cannot be attributed to biliary tract disease.

The administration of frequent blood transfusions to patients with chronic anemia might lead to hepatic dysfunction by two mechanisms: (1) the production of homologous serum hepatitis and (2) the production of secondary hemochromatosis as a result of the deposition of iron contained in the transfused blood. In the cases described transfusions were used sparingly, and only one patient had recognizable homologous serum jaundice. This patient displayed little evidence of residual hepatic damage. There was no clinical evidence that any of the patients developed hemochromatosis. While iron deposition in the liver was often prominent in the cases examined at autopsy, it was not regularly present. Cirrhosis in the 4 cases studied was thought not to be attributable to pigment deposition.

It is possible that hepatic dysfunction might be the result of the prolonged severe anemia. Other anemias present from early life and of such severity are rarely encountered. Pronounced evidence of liver disease is not commonly seen in individuals with chronic anemia. Paroxysmal nocturnal hemoglobinuria is a chronic hemolytic anemia which may exist for many years and be associated with extremely low hemoglobin levels. It is of interest that patients with this disease were observed to have considerably less evidence of hepatic dysfunction than the patients with sickle cell anemia (Table II). It seems unlikely that the hepatic disorder in sickle cell disease can be attributed solely to the anemia or to the hemolytic process *per se*.

It is well known that patients with sickle cell anemia may have striking clinical evidence of cardiac disease, with cardiac dilatation, impressive murmurs and electrocardiographic changes. Chronic congestive failure might account for the development of hepatic dysfunction in these patients. In the group of patients studied cardiac abnormalities were frequent and of marked degree. However, congestive failure was rarely encountered, occurring usually as a part of the terminal picture long after hepatic enlargement and dysfunction had been demonstrated. The rarity of cardiac failure in patients with sickle cell anemia has previously been noted (2, 9).

TABLE II

Liver function in five patients with the Marchiafava-Micheli syndrome (chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria)

CASE	HISTORY NUMBER	PATIENT (AGE, RACE, SEX)	HEMATOCRIT	LIVER SIZE	SERUM BILIRUBIN TOTAL (DIRECT)	SERUM ALBUMIN	SERUM GLOBULIN	CEPHALIN FLOCCULATION (0 TO +++)	THYMOL TURBIDITY	ALKALINE PHOSPHATASE	BSP RETENTION	PROTHROMBIN TIME	UROBILINOGEN IN URINE (0 TO ++++)	BILE IN URINE (0 TO +++)	GALL BLADDER X-RAY	DURATION OF ANEMIA
			%	cm.	mg./100 ml.	Gm./100 ml.	Gm./100 ml.		(units)	(units)	mg./100 ml.	sec.				
1	508246	D. G. 49 WF	13	0	9.0 (1.5)	5.1	1.4	0	1.8	3.4	0.7	—	+	0	0	18 years
			24	5	53 (28.8)					6.2			++++			
2	328547	D. W. 76 WF	33	4	1.7 (0.8)	4.7	2.7	—	—	3.9	tr.	—	0	0	—	6 years
			34		4.8 (2.4)											
3	267929	K. M. 46 WF	7	0	3.4 (1.5)	5.8	1.3	++	6.4	2.1	—	—	+	0	—	9 years
			39		2.2 (1.4)			+++	11.0							
4	537721	R. D. 30 CF	24	0	1.6 (0.8)	—	—	—	—	—	—	—	+	0	0	3 years
5	231582	H. R. 50 WF	21	0	1.8 (1.1)	3.7	2.0	0	7.8	3.3	0.2	17	0	0	+	15 years
			38	4	1.9 (1.1)	5.1	2.3		8.3	4.1	0.5		++			

No evidence was obtained that the liver disorder in these patients could be attributed to dietary factors. It is of interest that in spite of the chronic disability, alcoholism was not encountered in this group. Food intake was apparently adequate and definite evidence of dietary deficiency was not recognized.

Of the 50 patients examined 7 were known to have had syphilis. All of these had been adequately treated. Four received penicillin and three had been given heavy metal therapy. In no instance was there evidence that the syphilitic infecion or the antiluetic therapy was directly related to the hepatic disorder.

The dysfunction of the liver occurring in sickle cell anemia seems to be a specific feature of that disease and was readily accounted for by the pathological changes observed at autopsy in some cases although remaining more obscure in others. In the numerous reports considering the pathological aspects of fatal sickle cell disease, alterations of the liver have rarely been emphasized. Changes similar to some of those described in our report have been frequently mentioned (10–13). The sinusoidal engorgement by sickled red cells, erythrophagocytosis by the Kupffer cells, hemosiderosis, parenchymal atrophy and fatty infiltrations especially located in the central regions of lobules have all been described. Rarely, more serious evidence of liver cell damage has been noted as in the report by Ryerson and Terplan (11) who described "marked dissociation of liver cords, extensive necrobiosis, and interstitial fibrosis" in one of their cases. Although transfusions had been given this patient, the descriptions and photomicrographs were not characteristic of homologous serum hepatitis. Kimmelstiel (13) described massive necroses or infarcts in the liver of an 11 year old girl who had had no transfusions and he found no vascular occlusions. This child also had ischemic necroses of the kidney, brain, and gall bladder and Kimmelstiel assumed that the lesions were the result of acute vascular spasm. No vascular thrombi were seen in the livers of our cases but the large fresh necroses in five of our cases had the centralobular distribution so characteristic of generalized impairment of effective hepatic blood flow such as is seen in very severe acute anemia, shock, or severe chronic passive congestion. In fatal cases of sickle cell disease with the severe anemia and frequent terminal crises, it is surprising that more of these central necroses are not encountered. The focal necroses which we have described may also be attributed to local ischemia. The local shunting of hepatic sinusoidal blood flow as a result of some vascular spasm may theoretically be a factor but more obvious is the probable obstructive effect upon sinusoidal blood flow of the red cell engorged Kupffer cells "fixed" as they are in the hepatic sinusoids, as well as the important effect of intra-vascular sickling upon the viscosity of the blood. It seems altogether likely that these latter two mechanisms function in the liver of patients with sickle cell disease, especially when combined with the very severe anemia so often present, to result in a critical reduction of effective hepatic blood flow.

That marked functional manifestations may be discernible without the demonstration of severe morphological lesions by present histologic techniques, seems quite reasonable.

Other reports, which mention occasionally the occurrence of "interstitial fibrosis", have not included cases of definite cirrhosis. One might expect to find that our four cases of unequivocal cirrhosis would be of the central or "cardiac" variety. This was not the case. The areas of very broad scar in two cases suggest that these are probably post-necrotic cirrhoses. Two of the patients had previous transfusions, another had several liver injections, while the record of the fourth, the child of 6½ years, is missing. If liver cell regeneration is impaired in the presence of anoxia, diffuse scarring might result from repeated small necroses of liver cells although the normal liver probably can always repair such lesions without scarring. The recent focal necroses and old tiny areas of atrophy and early fibrosis seen in 5 of our cases are of interest in this respect. In one of these cases, in whom mitral stenosis was present, chronic cardiac failure may have contributed to but could not have caused the unusual and severe hepatic cirrhosis which also was not at all similar to post-necrotic scarring.

In the past it has been common practice to subdivide patients with sicklemia into those who have the asymptomatic sickle cell trait without anemia, and those with anemia. Patients have been encountered who at times appeared to have the sickle cell trait, yet at other times displayed symptoms characteristic of sickle cell disease (14). Itano and Neel (15) and Kaplan, Zuelzer, and Neel (16) have obtained evidence that multiple genes may be concerned with the production of the disease, and that these atypical variants may have a different genetic background than the more usual forms of sickle cell anemia. Eleven of the fifty patients in the present series were considered atypical. It is entirely possible that the genetic pattern of these patients was different from those with sickle cell anemia. While these patients had evidence of liver dysfunction, on the whole the disorder was milder. In contrast, asymptomatic individuals with the sickle cell trait showed no evidence of hepatic dysfunction.

SUMMARY

In a study of fifty patients with sickle cell anemia, clinical evidence of hepatic dysfunction was frequently encountered. In a few cases there was severe hepatic disease. The disorder of the liver seemed to be a specific manifestation of sickle cell anemia, and could not be explained on any other basis. Examination of the liver at autopsy of twenty-one cases of sickle cell anemia suggested that hepatic dysfunction probably can be explained by severe impairment of hepatic blood flow from the combined effects of anemia, the sickling of red cells in hepatic sinusoids and obstruction by Kupffer cells engorged by phagocytized red cells. The latter two processes are essentially unique to

sickle cell disease. There were morphological evidences of hepatic cell injury and unequivocal cirrhosis was seen in four cases.

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LEGEND

Figs. 1–10. Microscopic appearance of the liver at autopsy of patients with the hepatic disorder associated with sickle cell anemia.

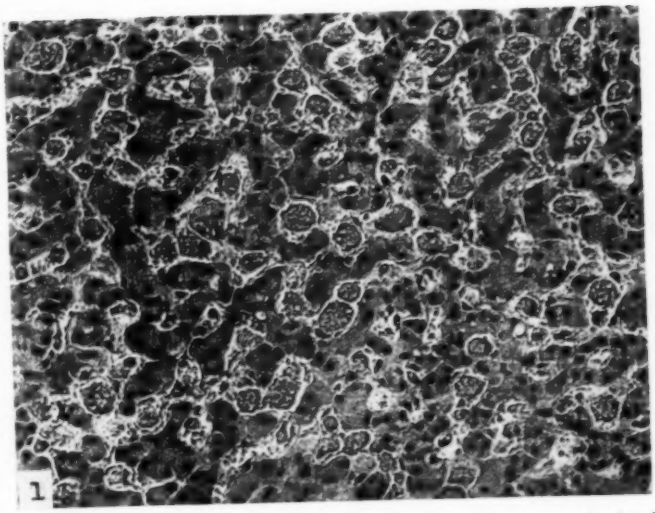

FIG. 1. Sinusoids with numerous Kupffer cells engorged with phagocytized red cells.

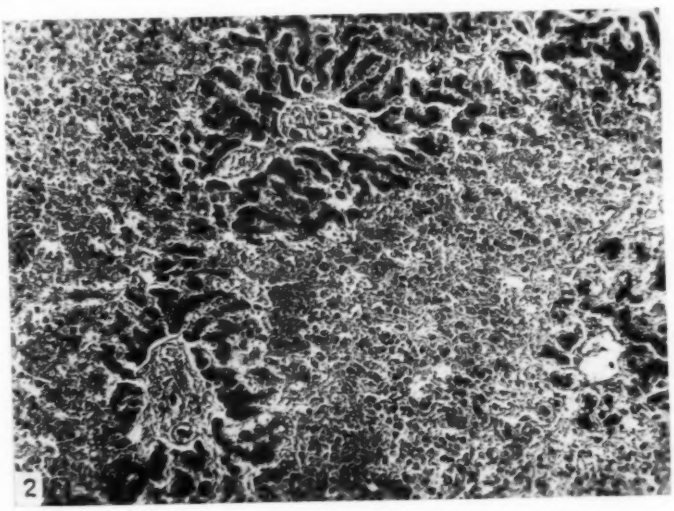

FIG. 2. Massive central necroses in patient dying in crisis.

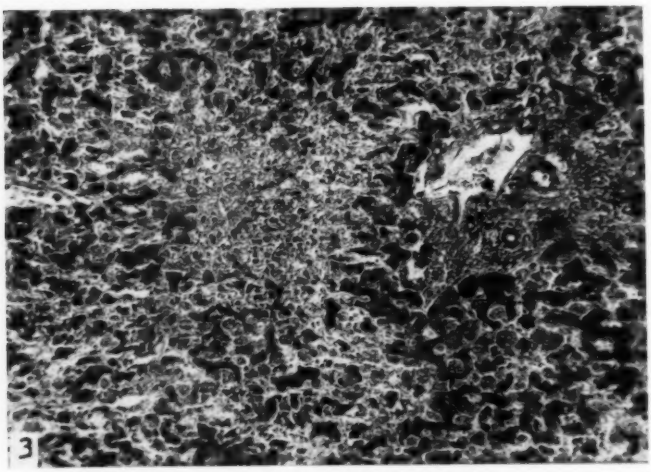

FIG. 3. Healing focal necrosis in mid-zonal area.

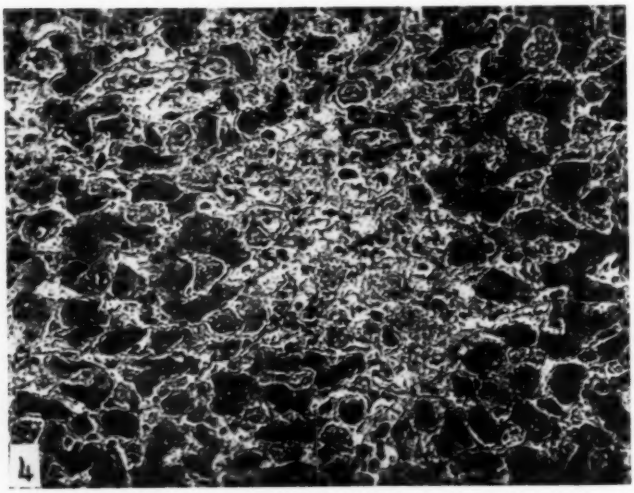

FIG. 4. Recent focal necrosis.

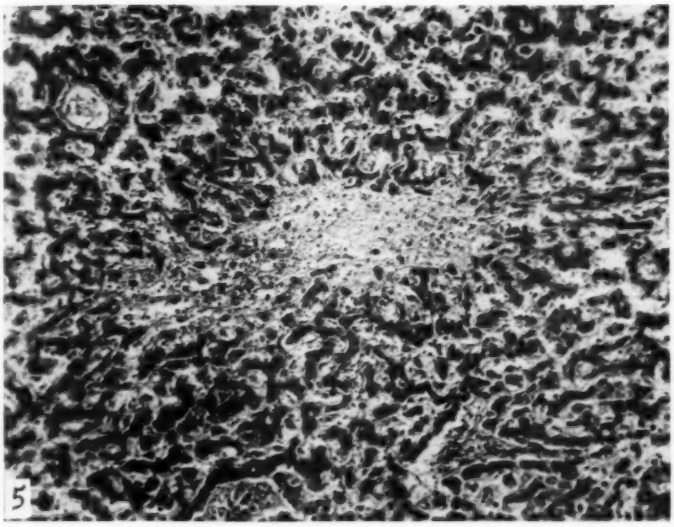

FIG. 5. Scars comparable in size and location to focal necroses.

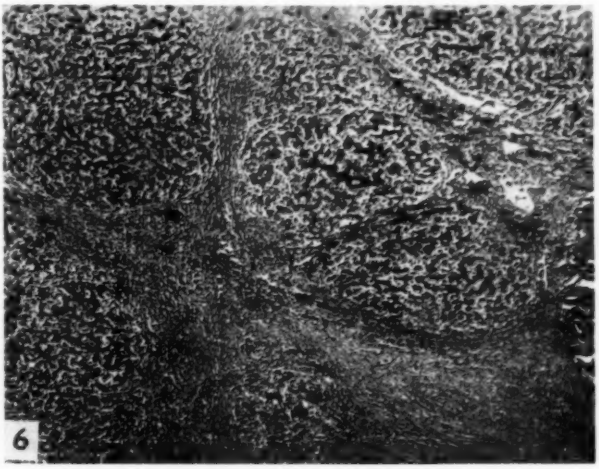

FIG. 6. Unequivocal cirrhosis of the liver.

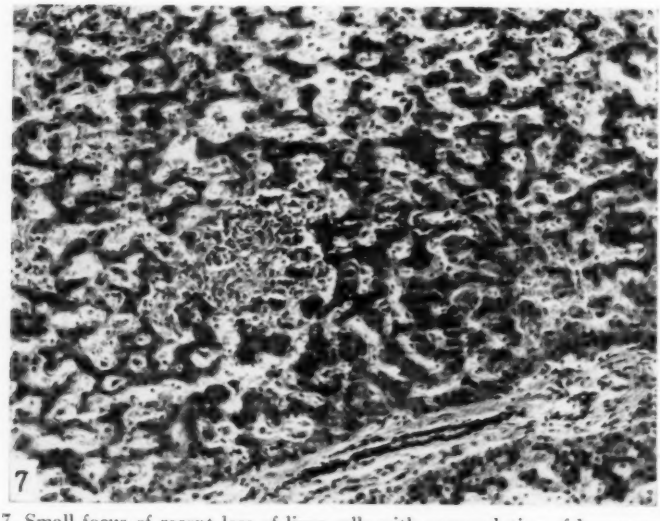

FIG. 7. Small focus of recent loss of liver cells with accumulation of leucocytes in midzonal area.

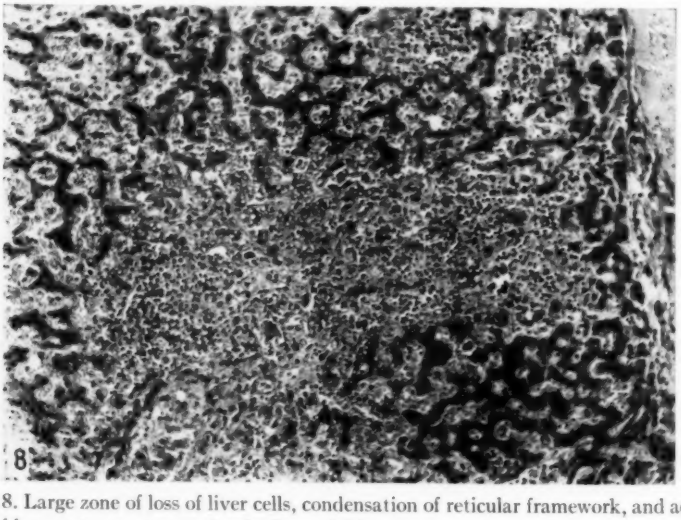

FIG. 8. Large zone of loss of liver cells, condensation of reticular framework, and accumulation of leucocytes near central vein (lower left).

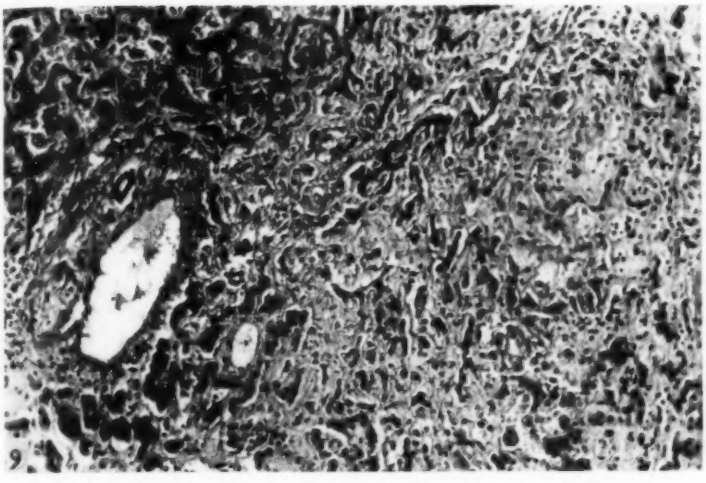

FIG. 9. Broad scars between liver cells, approaching periportal area.

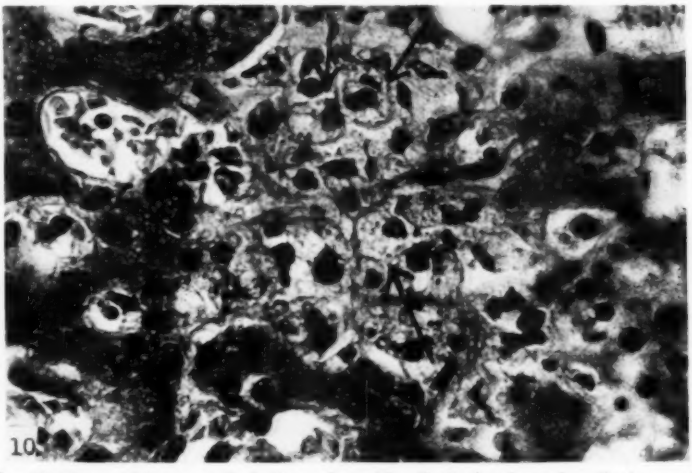

FIG. 10. Focus of foamy cells in liver sinusoids, displacing hepatic cells (arrows).

REVERSE DUCTUS

A Presentation of the Syndrome of Patency of the Ductus Arteriosus with Pulmonary Hypertension and a Shunting of Blood Flow from Pulmonary Artery to Aorta

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INTRODUCTION

The syndrome produced by simple patency of the ductus arteriosus has been thoroughly discussed by numerous authors (1, 2, 3). The purpose of this paper is to present a syndrome which differs clinically, physiologically and pathologically from that commonly associated with patent ductus arteriosus. This syndrome consists of patency of the ductus arteriosus in combination with sufficient pulmonary arterial hypertension to cause unoxygenated blood to enter the systemic circulation through the ductus. Because the ductus enters the aorta distal to the origin of the great vessels from the arch, venous blood is directed primarily to the lower extremities. Hence, in the lower extremities arterial oxygen saturation is lowered and cyanosis may be present.

Four patients with this syndrome have been studied at the Johns Hopkins Hospital. Two of the four patients were subsequently studied at autopsy. These two cases will be presented in detail; the two clinical cases will be reviewed briefly. The laboratory studies consisted of cardiac catheterization and simultaneous oxygen content and pressure determinations from the brachial and femoral arteries. Blood flows were calculated according to formulae described in a preceding communication (4). Pressures were measured by means of a strain gage manometer. Resistance in the pulmonary and systemic circulations was calculated by means of a simplified version of Poiseuille's formula (5). Pulmonary capillary pressures were not recorded. Therefore, the minimal pulmonary arteriolar resistance was calculated by assuming a maximal pulmonary vein pressure of 35 mm. mercury. The resistance thus obtained may be assumed to be minimal. The actual resistances were probably even higher. In addition, since vascular resistance is altered by changes in blood viscosity, true minimal pulmonary arteriolar resistance was obtained by correcting the value to a standard hematocrit (6).

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CASE REPORTS

Case 1: H. W. (H. L. H. No. A-68041) was a 36 year old white woman who was first seen in the Cardiac Clinic on January 18, 1949. Her chief complaints were cyanosis dating from birth, easy fatigability and dyspnea of increasing severity.

Past History and Present Illness: With the exception of mild cyanosis and dyspnea her childhood was uneventful. At about the age of puberty there was a marked increase in cyanosis, dyspnea and fatigability. Attacks of paroxysmal dyspnea occurred and polycythemia developed. There was no history of pneumonia, bronchitis or other pulmonary infections.

Physical Examination: The patient appeared well developed and well nourished. There was moderate cyanosis of the lips, mucous membranes and nail beds, and moderate clubbing of the nails. No difference in cyanosis between hands and feet was noted. Respirations were normal. Blood pressure was 115/85 mm. Hg. in the arms. The blood pressure in the legs was not determined. Radial and femoral pulsations were of good quality. The pulse rate was 80. Examination of the heart showed the maximal apical impulse in the fifth left interspace 1 cm. beyond the mid-clavicular line. No thrills were felt. The second heart sound over the base was split and accentuated. There was no definite systolic murmur. On subsequent examination, however, a faint systolic murmur was heard maximal in the third left interspace. There was a long, blowing, soft diastolic murmur heard over the entire precordium but maximal along the left sternal border. The lungs were clear. The liver and spleen were not felt. There was no peripheral edema.

Laboratory Findings: On fluoroscopy the right ventricle and right auricle appeared definitely enlarged. The main pulmonary artery segment was convex and pulsated vigorously. The right and left pulmonary arteries appeared unusually quiet. X-rays confirmed the fluoroscopic findings. (Figure 1).

Electrocardiograms revealed right axis deviation and right ventricular hypertrophy. The red blood cell count was 7.68 million per cu. mm. The hemoglobin was 19.5 grams; the hematocrit was 62.

During cardiac catheterization the catheter passed easily into the right auricle, the right ventricle and the main pulmonary artery. The catheter was not passed beyond the main pulmonary artery. Because of clotting, blood samples from the pulmonary artery were not obtained. Oxygen content determinations revealed no evidence of an arteriovenous shunt within the heart. Pressure recorded from the right ventricle was 137/36 mm. Hg. and from the main pulmonary artery was 128/96 mm. Hg. At rest, a sample of femoral arterial blood was found to be 98 per cent saturated. During exercise, arterial oxygen saturation, as determined by means of an ear oximeter, fell from 94 per cent to 87 per cent on one occasion and to 84 per cent on another. During exercise, oxygen consumption per liter of ventilation rose from 17.5 cc. to 20.5 cc. and, on another occasion, from 22.0 cc. to 26.0 cc. The essential data from cardiac catheterization are tabulated in Table I.

Impression: On the basis of the above findings, several possible conclusions were reached: (1) at rest there was no venous arterial shunt but with exercise a venous-arterial shunt appeared; (2) at rest there was no evidence of an intracardiac arteriovenous shunt; (3) the marked increase of pressures recorded from the right ventricle and the main pulmonary artery suggested an obstruc-

tion to the pulmonary circulation distal to the main pulmonary artery; (4) the fluoroscopic evidence of clear lung fields peripherally and the absence of pulsations in the right and left pulmonary arteries near the hilum suggested that the point of obstruction lay near the bifurcation of the main pulmonary artery; (5) a foramen ovale held open by high pressure in the right side of the heart was thought to be the pathway for the venous arterial shunt.

Course: On April 12, 1949, Dr. Alfred Blalock performed a left exploratory thoracotomy. A large patent ductus arteriosus was found. The blood flow in the ductus arteriosus seemed

FIG. 1. PA of chest—Case 1.
Note the clear lung fields, convex main pulmonary artery and slight cardiac enlargement.

to be from the pulmonary artery to the aorta, since a thrill was present in the aorta. There was no obvious coarctation of the aorta. During dissection of the ductus arteriosus, bleeding occurred from its posterior surface. This could be controlled with ease by pressure on the ductus. However, every time pressure was applied heart action ceased. After the third period of cardiac arrest it was impossible to reestablish heart action.

Permission for a post mortem examination through the incision was granted.

Autopsy: J. H. H. 21733 (Dr. R. P. O'Donnell) Examination was limited to the thoracic surgical incision. Three thousand cc. of dark liquid blood was found in the left pleural cavity with complete atelectasis of the left lung. The right atrium and ventricle were hypertrophied and dilated, the right ventricular myocardium measuring 1.0 cm. in thickness. The foramen ovale was anatomically closed. The wall of the dilated pulmonary artery was equal to, or

greater than, the thickness of the aortic wall and yellow arteriosclerotic plaques studded the intimal surfaces of both the main pulmonary artery and its larger branches. Two cm. distal to the pulmonary bifurcation, a patent ductus arteriosus opened into the left pulmonary artery. It measured 0.5 cm. in diameter at this orifice. Unfortunately, this area was damaged in the post mortem removal of the organs and therefore the detailed anatomy of the ductus could not be ascertained. No vegetations were seen on the cardiac valves nor at the pulmonary end of the ductus. The left pulmonary artery was so studded with arteriosclerotic lesions that no comment regarding the so-called "impingement plaque" could be made. Evidence

TABLE I

	H.W. #1	C. B. #2	R. H. #3	E. L. #4
Oxygen Content: (Vol. %)				
Superior Vena Cava	16.0	12.6	24.6	
Inferior Vena Cava				19.2
Right Auricle	15.43	11.9	23.5	19.2
Right Ventricle	15.13	11.67	23.6	19.9
Pulmonary Artery		clotted	23.2	19.9
Brachial Artery		20.0†(85.7%)	28.99†(95%)	23.5‡(91%)
Femoral Artery	25.5(98%)	13.49†(58%)	25.75†(84.5%)	17.7‡(70%)
				31.6
Oxygen Capacity: (Vol.%)	26.15	24.61	30.58	25.59‡
Pulmonary Blood Flow (cc./min./M2)	990	1250	2160	1130
Maximal Systemic Blood Flow (cc./min./M2)+	1025	6290	3960	6650
Venous-Arterial Shunt (cc./min./M2)	35	5040	1800	5520
Pressures: mm. Hg.				
Right Auricle	13/12	14/5	18/10	13/6
Right Ventricle	137/36	110/17	135/10	100/12
Pulmonary Artery	128/96	100/60	135/82	92/50
Brachial Artery	115/85*	121/84†	115/67†	110/78‡
Femoral Artery		137/64†	140/58†	108/68‡

* = By cuff.
† = Direct obtained the day following catheterization.
‡ = Direct obtained 19 months following catheterization.
\+ = Arteriovenous difference based on femoral artery and right auricular blood oxygen content. Therefore, the result is the maximal systemic blood flow.
( ) = Percentage Oxygen saturation.

of an "impingement" plaque in the aorta opposite the ductus was sought. Arteriosclerotic plaques about the aortic orifice of the ductus were seen but these were more prominent in this location than on the aortic wall opposite the opening of the ductus. The arch was otherwise free of arteriosclerosis as was the descending thoracic aorta; the latter portion actually appeared hypoplastic. The left ventricle seemed smaller than normal with a wall 1.0 cm. in thickness. The pulmonary veins entered a normal left atrium. The endocardium of both ventricular chambers was thin and transparent. Cross sections of the atelectatic lungs revealed very prominent vessels. Emphysematous blebs were encountered in both apical lung areas.

Microscopic: Significant histologic lesions were seen only in the pulmonary vessels. The largest pulmonary arteries presented subintimal accumulations of foam cells as well as intimal proliferation. The media of these vessels was normal. The medium-sized arteries, adjacent to bronchioles, often showed marked narrowing of their lumina by intimal proliferation which consisted of relatively acellular fibers which were arranged either eccentrically or concentrically. The internal elastic lamella was preserved and the media was usually normal although occasionally hypertrophied. Adventitial thickening about such vessels was sometimes apparent. Stains were negative for iron. Several such vessels which were seen in longitudinal section contained fresh or organizing thrombi which blended gradually with the old fibrous and hyaline intimal thickenings. Other vessels, smaller than the above but also located near bronchioles, contained typical thrombi. These occasionally occluded the vessel lumen but usually were recanalized which resulted in many small blood-filled channels. Many sections showed that these thrombi were lodged in branches which arose from those medium-sized muscular arteries that exhibited the intimal thickening. Furthermore, longitudinal sections of such lesions demonstrated that the intimal proliferation was often most prominent near the orifice of such a thrombosed vessel. The intimal fibrosis extended into the branch and down to the organized thrombus. The smooth muscle and lamellae of such thrombosed vessels showed fragmentation. Some of the recanalized lesions were enormous and indeed so large as to almost fill a low power field. Some resembled large and peculiar cavernous hemangiomata. Fresh thrombus material in different stages of organization was occasionally found in the lumina of these channels. At the periphery of many of these vascular lesions, most often about the recanalized thrombi, large, yet very thin-walled, blood-filled vessels were seen. No communication was ever seen between these vessels and the artery near which they were found. The smallest of pulmonary vessels were normal although these, including the alveolar capillaries, were often greatly dilated. Bronchial arteries also appeared normal.

Small focal scars were seen in the myocardium but no recent necroses were found Small hyaline plaques in the tiny myocardial arterioles were rarely present. No other vascular lesions were seen in the sections of the aorta or liver. Chronic passive congestion was not present. An infrequent hyaline thickening of the bronchial epithelial basement membrane was noted as was a small focus of fresh lipoid pneumonia. No pulmonary scars were present but atelectasis and emphysema were seen in different sections of lung.

Case 2: C. B. (No. 497330) This patient was a 29 year old woman who was first seen in April of 1949.

Past History and Present Illness: In every respect she was considered a normal child until the age of seven when, after running home from school, she was suddenly stricken with a severe precordial pain which radiated down the inner aspect of both arms, dizziness, weakness and a cold sweat. This attack lasted one-half hour and then subsided gradually. During subsequent years she suffered from many similar attacks, some of which were initiated by a tachycardia.

Cyanosis was first noted immediately after the onset of symptoms and thereafter steadily increased in severity. Exercise, fatigue, excitement or attacks of tachycardia caused an increase in cyanosis. Concomitant with increasing cyanosis was a steady decrease in exercise tolerance and increase in polycythemia. At the time of examination, exercise was limited to walking less than one block or climbing less than one flight of stairs.

Both the patient and her relatives repeatedly noticed that when cyanosis suddenly increased with exercise or tachycardia the cyanosis was more marked in both feet and the

left hand than about the face and right hand. This observation was corroborated at the time of cardiac catheterization when the patient became intensely cyanotic about the feet and left hand and developed precordial pain and pulmonary edema. At rest, however, the distribution of cyanosis was uniform.

For the past thirteen years the patient had noticed hoarseness, which was at first mild but gradually increased in severity. A chronic cough and frequent small hemoptyses occurred. She suffered from cardiac decompensation which on several occasions responded to treatment.

Physical Examination: On examination the patient appeared emaciated and chronically ill. There was moderate cyanosis of the lips and nail beds. At that time no difference was noted in the distribution of the cyanosis between the upper and lower extremities. Blood pressure in the arms was 140/100 mm. Hg. and in the legs 135/100 mm. Hg. Femoral pulsations were forceful but the brachial and carotid pulsations were difficult to feel. The heart seemed moderately enlarged to percussion. There was a coarse systolic thrill over the base of the heart and a long, loud, very harsh systolic murmur well localized in the second left interspace. Along the left sternal border there was a long, rather loud, blowing diastolic murmur which had the characteristic quality of the murmur of aortic or pulmonary insufficiency. The second heart sound at the base was sharp and split. There was no peripheral edema.

Laboratory Findings: The patient presented an extraordinary picture under the fluoroscope. The aortic valve was visible as a heavily calcified structure. The ascending aorta and aortic knob were calcified. In addition, the main, right and left pulmonary arteries showed calcification well out into the lung fields. The main pulmonary artery segment was very convex. The pulmonary arteries were large and marked pulsations were visible. The outflow tract of the right ventricle was enlarged. The right auricle was moderately enlarged. The left side of the heart appeared normal in size and configuration (Figure 2).

An electrocardiogram revealed right axis deviation, right ventricuar hypertrophy and prolongation of the A-V conduction time. The red blood cell count was 7.2 million cu. mm., the hemoblobin was 18 grams and the hematocrit was 80.

Cardiac catheterization revealed no evidence of an arteriovenous shunt within the heart. Pressures in the right ventricle and pulmonary artery were significantly elevated; i.e., 110/17 and 100/60 mm. Hg. The oxygen saturation of femoral arterial blood was 57 per cent. Simultaneous oxygen saturation and pressure determinations were made from the right brachial and femoral artery on the day following catheterization. Oxygen saturation of right brachial arterial blood was 85.7 per cent whereas saturation of femoral arterial blood was only 57.9 per cent and fell to 56.3 per cent with exercise. This difference indicated that venous blood entered the arterial circulation at a point distal to the origin of the right innominate artery.

Aortography to ascertain the presence or absence of coarctation of the aorta was considered but seemed contraindicated because of the rather critical condition of the patient.

Clinical Impression: The final clinical diagnosis in this patient was pulmonary hypertension and patency of the ductus arteriosus with a venous arterial shunt through the ductus. There was no definite evidence of coarctation of the aorta. In addition there was calcification of the great vessels with probable resultant coronary insufficiency. This patient was the only one in this series who was observed to have a marked difference in the degree of cyanosis between the upper and lower extremities.

Course: The patient was discharged from the hospital. Her condition remained unchanged until five weeks prior to her death in April, 1950. At that time she vomited an unknown quantity of blood. The hemoglobin was found to be 62 per cent. There was no more gastrointestinal bleeding and x-ray studies of the gastrointestinal tract at the Martha Jefferson Hospital were negative. The patient, however, complained of almost constant upper abdominal pain. She suddenly lapsed into coma and died three days later.

Autopsy: Martha Jefferson Hospital, #4714 (Dr. Gilmer).* The protocol received from Dr. Gilmer indicated that the heart weighed 400 grams with right ventricular hypertrophy and dilatation. The myocardium of the right ventricle measured 1.2 cm. The left measured 0.9 cm. A patent ductus 1½ cm. in diameter was found joining an aorta and pulmonary

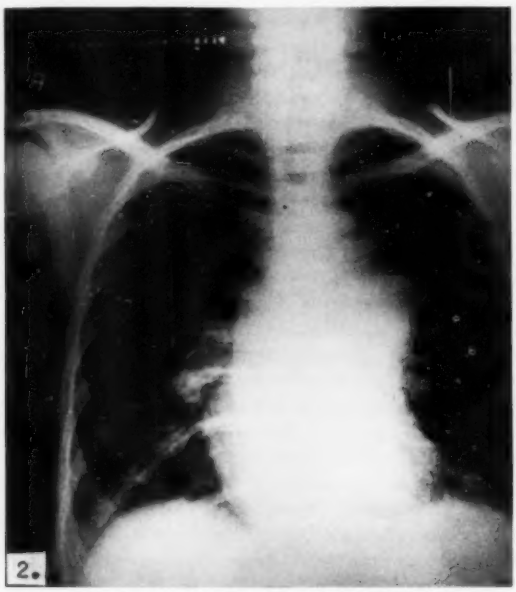
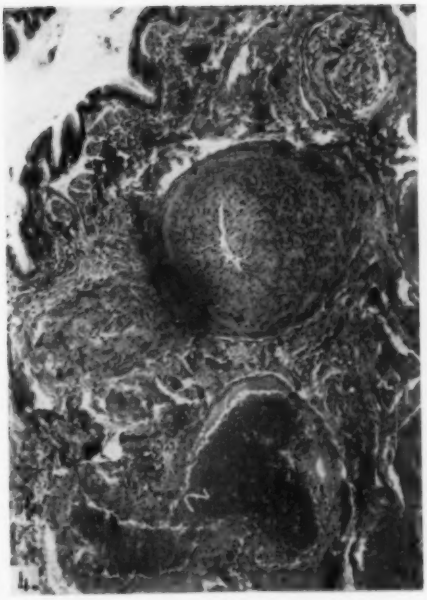


FIG. 2. PA of chest—Case 2.

Note the cardiac enlargement and the clacification of the aorta and both branches of the pulmonary artery.

artery which were closely adherent at this point. Very severe arteriosclerosis with calcification surrounded the aortic orifice of the ductus but did not involve the entire circumference of the aorta. The pulmonary artery showed enormous thickening of its wall as well as dilatation which attained a maximum of 11 cm. circumference. The main pulmonary arteries were involved by a very severe atheromatous intimal change which reduced the lumina of the superior branches to slit-like openings. The inferior branches were dilated, with intimal atheromata which were covered by organizing mural thrombi. Their lumina were also greatly

* We are indebted to Dr. Woodbury Perkins of Charlottesville, Virginia, for the details of her subsequent course and to Dr. Gilmer and Dr. Cash of the Martha Jefferson Hospital, Charlottesville, Virginia, for the autopsy findings.



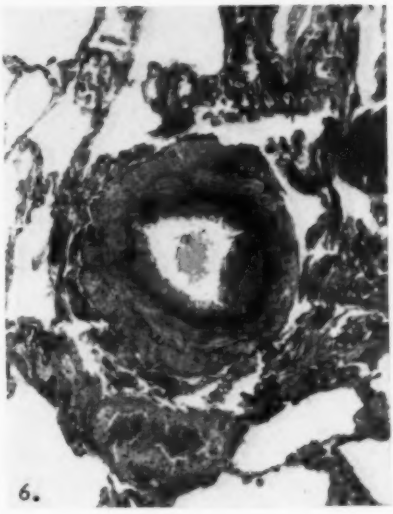
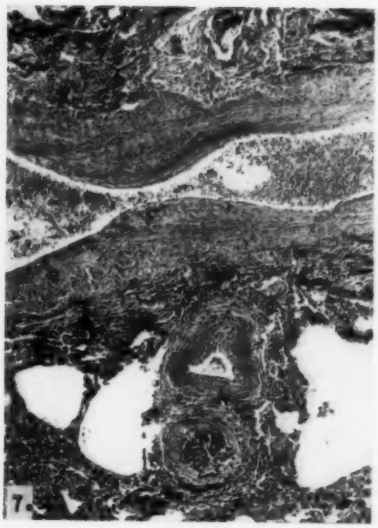


FIG. 3. Intimal arteriosclerosis of elastic pulmonary artery. Case 1.

FIG. 4. Severe arteriosclerosis or recanalized thrombosis muscular pulmonary artery with definite recanalized thrombus, upper right. Case 1.

FIG. 5. Recanalized thrombus in muscular pulmonary artery in elastic tissue stain. Case 1.

FIG. 6. Arteriosclerosis, muscular pulmonary artery in elastic tissue stain. Case 1.



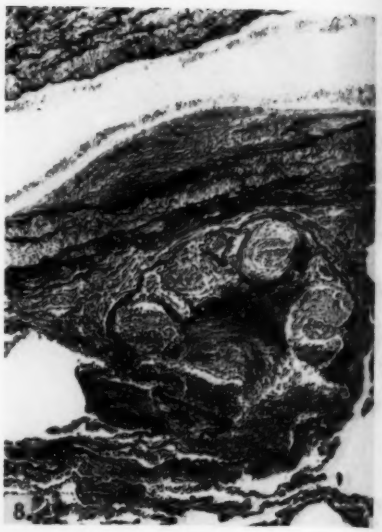


FIG. 7. Recanalizing thrombus in small pulmonary artery with arteriosclerosis in larger vessel. Case 1.

FIG. 8. Section cut deeper from same vessel as in Fig. 7 in elastic tissue stain. Case 1.

FIG. 9. Recanalized thrombus in branch of muscular pulmonary artery. Case 1.

FIG. 10. Recanalized thrombus in branch of muscular pulmonary artery with intimal plaques in larger vessel. Sinusoidal vessels surround lesion. Case 1.

narrowed. Arteries of the third and fourth order showed yellow intimal plaques. Little sclerosis was seen in the descending and abdominal aorta. Chronic passive congestion was prominent in the liver.

Microscopic: Study of microscopic sections received from Dr. Gilmer showed that the intima of the major pulmonary arteries was altered by very thick, fatty plaques in and beneath which calcifications had occurred. One section showed a huge, partially necrotic and organizing thrombus which covered such a plaque. The media of these pulmonary arteries resembled the aorta in thickness and structure. Medium and small pulmonary arteries showed marked intimal proliferation which often greatly narrowed their lumina. Other vessels, often smaller than the former, were occluded by completely organized hyaline, sometimes recanalized, thrombi. Some of the organizing and recanalizing thrombi formed large cavernous channels but others appeared as mural lesions. Around the margins of many of the above pulmonary vascular lesions, dilated blood-filled thin-walled vessels were seen and the smallest pulmonary vessels, including the alveolar capillaries, were enormously dilated. Emphysema was present.

The aorta showed intimal atheromata. Fine scars were found between hypertrophied muscle fibers in the right ventricle and septal wall. These were sometimes perivascular. The liver showed diffuse acute central necroses and the pancreatic acini lying distal to the islands were atrophic. The renal glomeruli were diffusely enlarged with thickening of the capillary basement membranes and slight increase of glomerular cellularity. A few glomerular arterioles revealed hyalinization of their walls. The femur marrow was hyperplastic.

Case 3: R. H. (H. L. H. No. A-81494) The patient was a 20 year old healthy-appearing male who was first seen in the Cardiac Clinic in 1933 at the age of 4.

Past History and Present Illness: A heart murmur had been noted at the age of 8 months. Examination in 1933 revealed a soft, poorly transmitted, systolic murmur maximal at the apex.

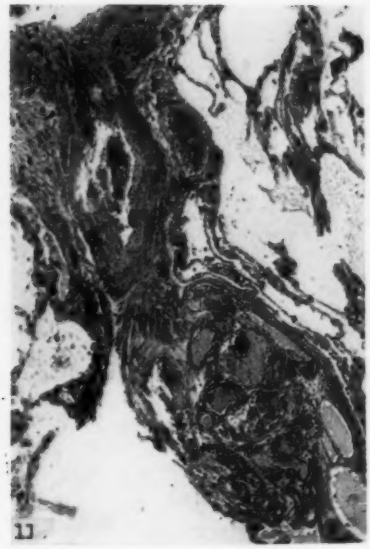
The patient was next seen in April, 1949. Until the age of 19 he had participated in all forms of sports and enjoyed excellent health. During his 19th year he noted distinct dyspnea on walking uphill or running the length of a basketball court.

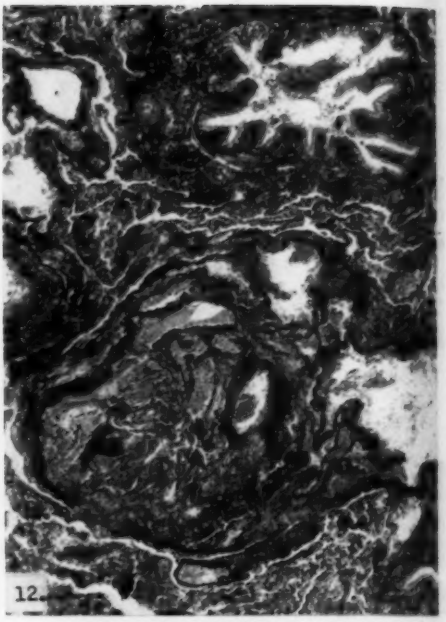
Physical Examination: The patient appeared well nourished and well developed. His color was ruddy. There was questionable cyanosis and clubbing of the extremities. The blood pressure in both arms was 112/70 mm. Hg. There was a grade 2 systolic murmur maximal along the left sternal border. The second heart sound was reduplicated and accentuated. Femoral pulsations were normal.

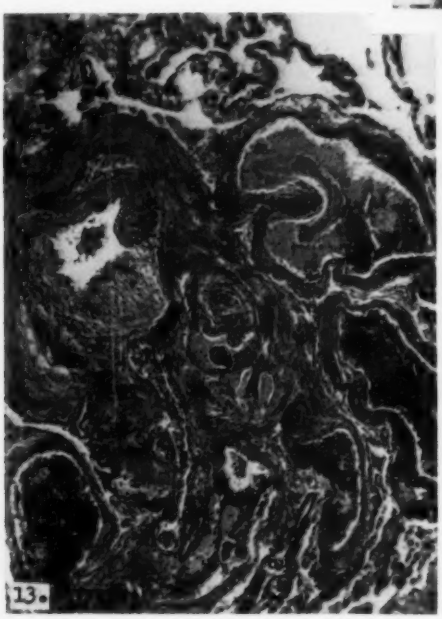
Laboratory Findings: On fluoroscopy the heart appeared normal in size and the lung fields appeared of normal vascularity. The main pulmonary artery segment was full. An electrocardiogram revealed right axis deviation and right ventricular hypertrophy. The red blood cell count was 8.45 million per cu. mm., hemoglobin 25 grams and hematocrit 71.

Cardiac catheterization revealed a pressure in the right ventricle of 135/10 and in the pulmonary artery of 135/82. There was no evidence of an arteriovenous shunt. With exercise, oxygen consumption per liter of ventilation rose. At the time of cardiac catheterization oxygen saturation, determined from femoral arterial blood, was 85 per cent. Arterial oxygen saturation determined by an ear oximeter was 99 per cent, remaining constant during exercise. Simultaneous arterial punctures were performed using the femoral and brachial arteries.* At rest, oxygen saturation of brachial arterial blood was 95 per cent, remaining con-

* It is not stated which brachial artery was used.







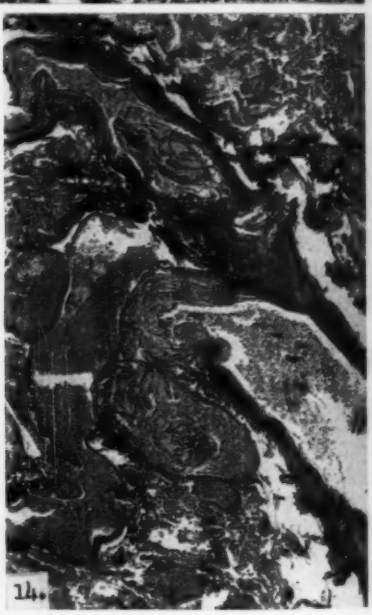
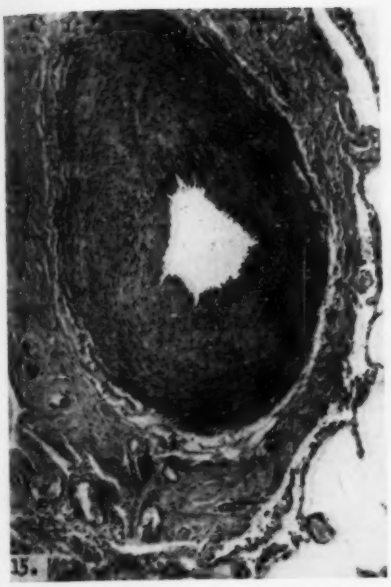


FIG. 11. Recanalized thrombus in branch of pulmonary artery. Case 1.

FIG. 12. Recanalized thrombus in muscular pulmonary artery in elastic tissue stain. Case 1.

FIG. 13. Recanalized thrombus. Case 1.

FIG. 14. Recanalized thrombi with large sinusoidal channels. Case 1.



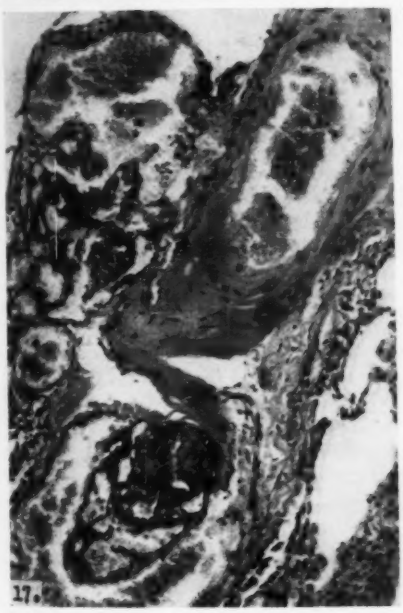


FIG. 15. Arteriosclerosis of muscular pulmonary artery. Case 2.

FIG. 16. Arteriosclerosis of muscular pulmonary artery with adjacent recanalized thrombi. Case 2.

FIG. 17. Recanalized thrombus. Case 2.

FIG. 18. Recanalized thrombus in tiny pulmonary artery with adjacent sinusoidal vessels. Case 2.

stant with exercise. At rest, oxygen saturation of femoral arterial blood was 84.5 per cent, falling to 80.5 per cent with exercise.

Intra-arterial aortography was performed to rule out a coarctation of the aorta. A catheter was introduced through the left ulnar artery and the tip was placed in the ascending aorta. Diodrast was then injected. A film taken after the injection revealed a good concentration of diodrast in the ascending aorta. The arch of the aorta and descending thoracic aorta were poorly visualized. There was no gross evidence of a coarctation of the aorta. A patent ductus arteriosus was not demonstrated nor was there any evidence of the dye in the peripheral lung fields.*

Impression: Pulmonary hypertension and patency of the ductus arteriosus with a venous arterial shunt through the ductus were considered the most likely diagnosis. The patient was discharged from the hospital.

Case 4: E. L. (H. L. H. No. A-55168) This patient was a 30 year old woman, first seen in the Cardiac Clinic in May, 1947. She was again seen in February, 1948, and September, 1949.

Past History and Present Illness: Her history was one of increasing cyanosis, dyspnea and easy fatigability dating from birth. At the time of her last visit (1949) she was able to walk only two blocks. In 1940 a cough developed and there was one episode of hemoptysis.

Physical Examination: The physical findings were essentially unchanged from 1947 to 1949. The patient appeared fairly well developed and well nourished. There was moderate cyanosis and clubbing. The blood pressure in both arms was 90/70 mm. Hg. and in the legs was 130/90 mm. Hg. The dorsalis pedis and femoral pulsations were bounding in character. The brachial and radial pulsations were normal. The heart did not appear to be enlarged clinically. There was a blowing diastolic murmur maximal in the second left interspace. There was a short, harsh systolic murmur heard at the base of the heart to the left of the sternum. The second heart sound was accentuated and slightly split.

Laboratory Findings: Red blood cell count was 9.0 million per cu. mm., hemoglobin 21.0 grams, hematocrit 74. An electrocardiogram showed right axis deviation and moderate right ventricular hypertrophy. Fluoroscopic examination revealed increased vascularity of the lung fields and a full pulsating main pulmonary artery. The right ventricle appeared moderately enlarged. X-rays confirmed the fluoroscopic findings.

Angiocardiography was done May, 1947. The right auricle and right ventricle appeared normal. The main pulmonary artery was dilated. In the second film the descending aorta was visualized momentarily and faintly in the lateral projection. The distal branches of the pulmonary artery were never well visualized. The left side of the heart and the ascending aorta appeared 14 seconds after injection. There was no evidence of coarctation of the aorta.

Cardiac catheterization was carried out in May, 1947, and January, 1948. There was no evidence of an intracardiac arteriovenous shunt. The pressure was elevated in the right ventricle (100/12 mm. Hg.) and in the pulmonary artery (92/58 mm. Hg.). With exercise, oxygen consumption per liter of ventilation rose slightly in 1947 (25.0 cc. to 26.0 cc.) and fell slightly in 1948 (23.7 cc. to 22.0 cc.). In 1949 simultaneous oxygen saturation determinations and pressure readings were made from the femoral artery and the right brachial artery. Oxygen saturation in the sample of blood from brachial arterial blood was 91 per cent and the pressure in this artery was 100/78 mm. Hg. Oxygen saturation in the sample of blood from femoral arterial blood was 70 per cent and the pressure was 108/66.

* We are indebted to Dr. Robert Sloan for this interpretation.

Final Diagnosis: The final diagnosis was pulmonary hypertension, patency of the ductus arteriosus, with the blood flow from pulmonary artery to aorta. The patient was discharged.

DISCUSSION

In 1944 Chapman and Robbins (7) reported the case of a 37 year old man, dyspneic and cyanotic from the age of 29. A systolic murmur was heard in the fourth left interspace, occasionally accompanied by a diastolic blow. Polycythemia was present. X-rays revealed a convex pulmonary conus and increased vascularity of the lung fields. There was right axis deviation in the electrocardiogram. Arterial oxygen saturations were repeatedly recorded in the neighborhood of 75 to 80 per cent. The patient died during operation for femoral vein ligation. Autopsy revealed a markedly hypertrophied right ventricle, a large patent ductus arteriosus and marked arteriosclerotic changes in the large and medium-sized pulmonary arteries with recanalized thrombi of small pulmonary arteries. The authors felt that the cyanosis and polycythemia were due primarily to "pulmonary factors" though some degree of venous arterial shunt through the ductus could not be excluded. Evidence for this conclusion was the rise in arterial oxygen saturation from 82 to 100 per cent in 30 minutes with inhalation of 80 per cent oxygen. It was, however, not stated whether arterial oxygen saturation was determined from femoral or brachial arterial blood samples

In 1947 Douglas, *et al.* (8) reported a 22 year old patient with a patent ductus in whom the typical diagnostic murmur was absent and cyanosis and dyspnea of 12 years duration were outstanding signs. Evidence of cardiac failure had been present for 18 months. The heart was enlarged. There was a soft diastolic and harsh systolic murmur maximal in the third and fourth left interspaces. Polycythemia was present. The electrocardiogram revealed right axis deviation. Cardiac decompensation progressed and the patient died. Post mortem examination revealed marked hypertrophy and dilatation of the right ventricle. There was a widely patent ductus arteriosus. A thickened, grey, intimal plaque was present in the aorta opposite the orifice of the ductus arteriosus. A similar plaque was not noted in the pulmonary artery. This finding suggested that blood flow through the ductus arteriosus had been directed from the pulmonary artery to the aorta. There were marked changes in the intima and media of many of the small arteries of the pulmonary bed. Recanalized thrombi in pulmonary arteries were also found. Because of endocardial thickening in the left ventricle, the authors postulated that flow through the ductus arteriosus in early life had been directed from the aorta to the pulmonary artery. They believed that secondary changes in the lungs resulted in pulmonary hypertension and a reversal of blood flow through the

ductus arteriosus. They advocated early ligation of the ductus to prevent this sequence of events.

In 1950 Johnson, *et al.* (9), reported the case of a 42 year old woman with a history of dyspnea dating from early childhood and signs of progressive cardiac decompensation. The heart was enlarged. There was a soft apical systolic murmur and a harsh diastolic murmur maximal in the third and fourth left interspaces. There was a mild polycythemia. The electrocardiogram revealed right axis deviation and evidence of hypertrophy of the right ventricle. X-ray examination revealed enlargement of the right ventricle and of the pulmonary arteries. Cardiac catheterization revealed a rise in oxygen content between the right ventricle and the pulmonary artery suggesting an arteriovenous shunt distal to the heart. The systolic, diastolic and mean pressures recorded from the pulmonary artery were very similar to the pressures recorded from the brachial artery. Simultaneous arterial oxygen saturation determinations from brachial and femoral arterial blood were found to be 91 and 85 per cent respectively. The patient died on the fifth post-operative day following ligation of the ductus. Autopsy findings revealed hypertrophy of the right ventricle, a window-type large ductus arteriosus only partially occluded by the surgical ligature, "jet" lesions opposite the orifice of the ductus arteriosus both in the aorta and pulmonary artery, atherosclerosis of the large pulmonary arteries, and moderate thickening of the media and intima of the small pulmonary arteries. No mention of the presence of pulmonary arterial thrombi was made. The authors postulated that this patient late in the disease developed an intermittent reversal of blood flow through the ductus due to an increasing pulmonary hypertension. They believed that the pulmonary hypertension probably was a result of a large arteriovenous shunt through the patent ductus.

There are striking similarities between the cases presented here and those reported in the literature. In our patients, cyanosis was noted at birth in two cases (Case 1 and Case 4), at 7 years (Case 2) and at the time of examination at the age of 20 in Case 3. Cyanosis was noted in Douglas' patient at 22, in Chapman's patient at 32, and in Johnson's patient at 42. In only one patient, (C. B., Case 2) was there a distinctive distribution of cyanosis.

Dyspnea was a prominent symptom in all patients. It was progressive and severe. Cough and hemoptysis were cardinal complaints in Case 2 and Case 4 in our series and in Chapman's patient. Right heart failure was present in the cases reported in the literature and in the second patient in this series.

Physical signs were similar. Pulse pressures were normal. The heart was only moderately enlarged. In five patients a short, harsh systolic murmur and a long, blowing diastolic murmur were present.

Fluoroscopic examination revealed enlargement of the right ventricle, prominence of the main pulmonary artery segment and, in five of the six cases,

large, moderately pulsatile, pulmonary arteries. The electrocardiogram revealed right axis deviation and, in those in whom precordial leads were taken, hypertrophy of the right ventricle.

PHYSIOLOGICAL CONSIDERATIONS

Douglas, Chapman and Robbins, and Johnson postulated that the pulmonary vascular changes were secondary to the markedly increased pulmonary blood flow resulting from a large arteriovenous shunt through a patent ductus. Catheterization studies in Johnson's patient suggested that the blood flow was primarily from the aorta to the pulmonary artery and that a reversal of blood flow was only intermittent. The findings in two of the four patients in our series suggest that increase in resistance in the pulmonary vascular tree was present very early in life and was not the end result of a long-standing postnatal arteriovenous shunt. These two patients were cyanotic from birth. In fetal life the flow of blood through the ductus arteriosus is directed from the

TABLE II

Mean Pressures: Mm. Hg

	PULMONARY ARTERY	BRACHIAL ARTERY	FEMORAL ARTERY
Normal	6–15	110–70	110–70
H. W. #1	107	95–109*	?
C. B. #2	73	96	88
R. H. #3	100	83	85
E. L. #4	69	89	79–80

* Obtained by cuff.

pulmonary artery to the aorta. The presence of cyanosis throughout life suggests that venous blood continued to flow from the pulmonary artery to the aorta. A physiological explanation for the direction of this blood flow may be found in a consideration of the pressure relationship between the greater and lesser circulations.

A mean pulmonary artery pressure of 15 mm. Hg. or lower is considered normal. The mean pulmonary arterial pressure in our four patients was 107, 73, 100, and 69 mm. Hg., respectively. These values represent a very marked increase above normal. In each instance, the mean pulmonary artery pressure was very close to the mean pressure in the brachial and femoral artery* (Table II).

It has been shown that an elevation of mean pressure within the pulmonary

* An exact comparison of mean pressure in the pulmonary artery and the systemic artery is not possible since direct determinations were obtained on different days. In addition, pulmonary artery pressures were obtained with the patient at rest and well sedated. Systemic artery pressures were obtained without sedation.

artery can be produced by two mechanisms: (A) Fixation of pulmonary arteriolar resistance and (B) an increase in the pulmonary vein pressure because of impaired drainage into or through the left side of the heart (10). Increase in pulmonary vein pressure can be ruled out in Case 1 and Case 2 since at autopsy there was no evidence of obstruction of the flow of blood through the pulmonary veins and left side of the heart. Similarly in the cases reported in the literature there was no evidence of such obstruction. There was, however, evidence of increased pulmonary vascular resistance in Case 1 and Case 2 as demonstrated by gross and microscopic study of the lungs. These findings were similar to those of Chapman, Johnson and Douglas. Table III illustrates that in

TABLE III

Reverse Ductus

	MEAN PULMONARY ARTERY PRESSURE	MAXIMAL PULMONARY VEIN PRESSURE	PULMONARY ARTERY INDEX	VISCOSITY RATIO	MINIMAL PULMONARY ARTERIOLAR RESISTANCE CORRECTED FOR HEMATOCRIT
	Mm. Hg		*L/mm./M2*		
H. W. #1	107	35	.990	1.38	54.3
C. B. #2	73	35	1.250	2.5	12.2
R. H. #3	100	35	2.160	1.76	17.0
E. L. #4	69	35	1.130	1.94	15.7

*"Typical" Patent Ductus Arteriosus**

	MEAN PULMONARY ARTERY PRESSURE	PULMONARY ARTERY INDEX	MAXIMAL PULMONARY ARTERIOLAR RESISTANCE
	Mm. Hg	*L/mm./M2*	
C. S. #1	25	3.860	6.45
A. B. #2	19	4.900	3.88
F. W. #3	44	17.350	2.52
A. T. #4	26	11.200	2.32
L. W. #5	17	9.550	1.78

* Results from proven cases of uncomplicated patent ductus arteriosus.

our other two patients (Case 3 and Case 4) the minimal pulmonary resistance was elevated.

The pulmonary artery pressure, calculated pulmonary artery blood flows, and pulmonary vascular resistances obtained in these patients are totally different from those obtained in patients with uncomplicated patency of the ductus arteriosus. Indeed, the minimal pulmonary arteriolar resistance in our patients is far greater than the maximal pulmonary arteriolar resistance in patients with simple patency of the ductus (Table III).

If the blood in the aorta above the level of the ductus arteriosus is fully saturated, the blood returning to the left side of the heart must be fully saturated. Under such circumstances, there can be no interference with the oxy-

genation of blood in the lungs, as suggested by Chapman and Robbins. Oxygen saturation of brachial arterial blood was 95 per cent in Case 3 and 90 per cent in Case 4, indicating no interference with oxygenation of blood in the lungs. In Case 2, oxygen saturation of right brachial arterial blood was 85.7 per cent, which suggested either an interference with normal oxygenation in the lungs, a shunting of unoxygenated blood via intrapulmonary shunts into the systemic circulation, or that venous blood passed through the ductus into the aorta and refluxed proximally into the right brachial artery.

In contrast to the high oxygen saturation obtained from samples of right brachial arterial blood, the oxygen saturations of blood samples taken from the femoral artery were definitely below normal; i.e., 84.5 per cent at rest and 80.5 per cent with exercise in Case 3, 70 per cent in Case 4, 57 per cent in Case 2 (Table I). This finding presents strong evidence that the primary source of femoral arterial unsaturation is not diffusion difficulty within the lungs, nor intrapulmonary arteriovenous shunts, but is the shunting of venous blood from the pulmonary artery through the ductus arteriosus to the descending aorta.

The total volume of blood flow (measured in cc. of blood/min/M2) which passes through a patent ductus arteriosus is dependent upon the size of the ductus and relative resistances in the pulmonary and systemic vascular beds. The flow is directed from the aorta to the pulmonary artery in patients with uncomplicated patent ductus arteriosus because normally the resistance in the systemic circulation is higher than that in the pulmonary circulation. Except in Case 2, the resistance in the pulmonary vascular bed in the group of patients presented in this report was equal to or greater than that usually found in the systemic circulation. In Case 2, the pulmonary artery pressures closely approximated the systemic pressures and clotting of blood was noted in the catheter which may have reduced the actual pressure. Hence, it is reasonable to suppose that blood might flow through the ductus arteriosus from the pulmonary artery to the aorta.

In the four patients under discussion an exact determination of relative resistances, relative pressures, and total blood flow through the ductus arteriosus cannot be made for the following reasons. First, as previously mentioned, although pressure determinations in the brachial and femoral artery were simultaneous, the measurement of the pressures in the pulmonary artery were not recorded simultaneously with those in the systemic circulation. Hence, we can only state that mean pressures in the pulmonary, brachial, and femoral arteries are about equal. Second, due to the existence of two values for arterial oxygen content (proximal and distal to the ductus) and of a different oxygen uptake for the upper and lower portion of the body, calculations for systemic blood flows and systemic resistances are not possible.

It is worthy of note that all of these patients did well during childhood but became progressively more incapacitated in early adult life. Several factors may account for this sequence of events. First, secondary changes in the pulmonary bed may be progressive, leading to further reduction in the cross sectional area of the total available pulmonary vascular bed and, hence, an increase in pulmonary hypertension. Concomitantly, multiple small arteriovenous shunts may develop. Both an increase in pulmonary vascular resistance and the development of small arteriovenous shunts increase cyanosis, the first by increasing the venous-arterial shunt through the ductus arteriosus and the second by increasing intrapulmonary arteriovenous shunts.

A second factor to be considered is the effect of pulmonary anoxia on pulmonary resistance. There is considerable evidence to show that acute pulmonary anoxia leads to an increase in pulmonary vascular resistance (11, 12). One might well hypothesize that this effect of anoxia ultimately terminates in a cycle. The increase in the pulmonary resistance may lead to an increase in the venous-arterial shunt, increasing anoxemia and perhaps causing a further increase in pulmonary vascular resistance. Indeed, it may be postulated that the effect of breathing 80 per cent oxygen in Chapman's case was to decrease pulmonary resistance. This, in turn, may have decreased or abolished the venous-arterial shunt through the ductus arteriosus and, consequently, led to a rise in arterial oxygen saturation to normal. We have observed the same rise in peripheral oxygen saturation in a patient with pulmonary arteriovenous aneurysms (13).

Third, the heavy load placed on the right ventricle by the high pulmonary vascular resistance may result in failure of the right side of the heart. Failure was present in three cases reviewed from the literature and in our Case 2.

In our opinion, the ligation of the ductus arteriosus in this syndrome when the high pulmonary vascular resistance becomes fixed and irreversible is contraindicated because the ductus arteriosus acts as an escape valve. Blood is shunted from an area of high resistance in the lungs to an area of lower resistance in the systemic circulation. In the patients studied at autopsy, the presence of narrowed, thickened arteries in the lungs and of normal arteries throughout the rest of the body suggests that resistance has become less variable in the pulmonic circulation than in the systemic circulation. Support of this theory is found in the small increase in pulmonary blood flow with exercise as determined by the measurement of the oxygen consumption per liter of ventilation. The increased work required of the right ventricle by the high pulmonary resistance eventually caused right ventricular dilatation and failure. The presence of a patent ductus arteriosus and a lower variable systemic resistance would combine to act as an escape valve and reduce the work of the right ventricle. Whether acute right-sided dilatation occurred terminally in our first patient (Case 1) is not known. This seems to us to be the most likely cause for

the sudden and immediate cessation of cardiac action when the ductus arteriosus was clamped. On the other hand, operation prior to the development of irreversible pulmonary vascular changes might prevent the sequence of changes noted in this series.

PATHOLOGICAL CONSIDERATIONS

The pulmonary vascular lesions found at post mortem examination in two of these patients appear sufficiently widespread and severe to have contributed to the pulmonary hypertension demonstrated during life. It is still impossible to know whether such lesions were the only or original cause of that hypertension. Furthermore, it is difficult to explain the pathogenesis of the lesions themselves.

The intimal fatty plaques in the largest or elastic pulmonary arteries are widely recognized as being a result of pulmonary hypertension, whatever its origin. This lesion, however, is rarely found among patients dying with patent ductus arteriosus, even in those showing right cardiac enlargement. While 32 of 57 cases reported by Keys and Shapiro (14) had right ventricular hypertrophy, only two showed arteriosclerotic plaques of the major pulmonary vessels and only one of Welch and Kinney's (15) series of 25 cases had this change.

Lesions of the smaller muscular arteries, somewhat similar to those found in our two patients, were described by Chapman (7), Douglas (8), and Johnson (9). The significant lesions described in all of these cases including our own are marked intimal proliferation of the medium and small pulmonary arteries, muscular hypertrophy of these vessels, the presence, except in Johnson's case, of organizing or organized thrombi in the pulmonary arteries, and the absence of changes in the minute pulmonary vessels lying in the walls of the air spaces. In our cases, the excessive intimal proliferation and the many thrombi were most prominent. It must be emphasized that old, completely organized mural thrombi or centrally recanalized thrombi may be indistinguishable from primary arteriosclerosis, even with special stains. Their origin may be suggested only by their association with all stages of organizing thrombi as were present in our two cases (16).

Bland or septic thrombi are common in patients with bacterial infection of the patent ductus arteriosus but unusual in uncomplicated cases. One patient reported with severe pulmonary arteriosclerosis was thought to have a healed endocarditis (7) and one of our patients had massive mural thrombi in the main pulmonary artery to explain the source of the small pulmonary vascular thrombi. The final clinical course of this patient was undoubtedly altered by the massive thromboses of the major pulmonary arteries which appeared largely to be terminal events. Possible sites of origin of thrombi include eddy currents set up in the area of an arteriovenous shunt as well as the chambers of a failing heart. Cardiac failure had been present in our Case 2 and had also occurred in

Douglas' patient. Castleman (17) has reported a patient who, in the absence of cardiac lesions, suffered repeated pulmonary emboli from a leg vein thrombosis and died with a cor pulmonale and recanalized pulmonary vascular thrombi which presented a similar appearance to those seen in our two cases. In both autopsied cases the location of the thrombi in the proximal portion of branches of small pulmonary arteries also suggests an embolic origin.

Other malformations leading to a large pulmonary blood flow also are associated with pulmonary vascular lesions consisting of intimal proliferation, medial hypertrophy and organizing thrombi. Such have been seen in Eisenmenger complex (18), coarctation of the aorta associated with patent ductus arteriosus (19), and true or pseudo-truncus arteriosus. In an infant only six months old with the latter malformation we have seen numerous fresh and old thrombi, many of which resembled a primary arteriosclerotic process. It must be noted that if intimal fibrosis occurred initially, perhaps as a consequence of enormous pulmonary blood flow or of pulmonary hypertension, mural thrombi might develop on such altered endothelial surfaces to produce the pathological picture which has been seen.

Finally, functional spasm of small pulmonary arterioles (10) may have initiated all changes leading to pulmonary hypertension, the shunt from right to left, and the development of cyanosis and polycythemia. The latter condition, of course, is prone to be associated with thrombus formation. Such a source of increased pulmonary resistance dating from birth more readily explains the cyanosis which appeared at birth in several of our patients. Even if muscular spasm of these vessels did initiate the hypertension and its consequences, there can be no doubt that the morphological changes found in the pulmonary vessels of our patients greatly increased the vascular resistance and caused it to become constant and irreversible. The theory of a persistence of the fetal type of thick-walled, narrow-lumened vessels stated by Edwards cannot be proven or disproven but the vascular obstructions seen in our two autopsied cases seem to be largely a result of intimal fibrosis and thrombi.

In the absence of more precise knowledge concerning intrapulmonary arteriovenous shunts, the effect of increased pulmonary flow on the pulmonary vascular bed, and the occurrence of a persistent pulmonary hypertension resulting from arteriolar spasm, the pathogenesis of the pulmonary hypertension and of the pulmonary vascular lesions in these cases remains obscure. Perhaps they represent cases of multiple congenital defects in which patency of the ductus arteriosus is combined with primary pulmonary hypertension.

CONCLUSIONS

The four patients presented here and the three cases reviewed from the literature suggest that the entity of pulmonary hypertension combined with

patency of the ductus arteriosus and a venous arterial shunt through the ductus arteriosus presents a distinctive clinical syndrome. The clinical history includes cyanosis, usually early in onset but usually not severe in degree until the second or third decade; severe dyspnea, increasing fatigability, and decreasing exercise tolerance. Physical examination reveals moderate cyanosis, a relatively small heart, usually a systolic and diastolic murmur maximal along the left sternal border, and an accentuated pulmonary second sound. If present, the appearance of more marked cyanosis in the lower extremities than in the upper extremities is very suggestive. On fluoroscopy the right side of the heart is enlarged. The main pulmonary artery segment is full and actively pulsating. The electrocardiogram reveals right axis deviation and right ventricular hypertrophy. There is oxygen unsaturation of femoral arterial blood, polycythemia and an elevated hematocrit.

The diagnosis may be substantiated by simultaneous brachial and femoral arterial oxygen determinations and pressure recordings. The oxygen content of the femoral arterial blood is significantly lower than that of the brachial arterial blood. Cardiac catheterization reveals a marked pulmonary hypertension and no evidence of an intracardiac arteriovenous shunt. Physiological studies indicate that cyanosis is primarily due to a venous arterial shunt through the ductus arteriosus.

Surgical closure of the ductus arteriosus prior to the development of extensive irreversible pulmonary vascular changes is highly desirable. After sufficient irreversible changes have occurred to cause an over-all reversal of blood flow through the ductus with blood flow from pulmonary artery to aorta, surgical closure is contraindicated, inasmuch as the ductus acts as an escape valve and reduces the work of the right ventricle.

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AN ELECTRON MICROSCOPE STUDY OF THE GIANT CHROMOSOMES OF DIPTERA BY MEANS OF SECTIONS

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In order to utilize fully the resolving power of the electron microscope in the study of cell structures, three primary conditions must be fulfilled. First, the cells must be fixed in a manner that will preserve the morphology of the living state. Second, they must be cut thin enough to allow penetration of the electron beam; and third, the cutting should produce a minimum of mechanical dislocation. In this study of the giant chromosomes of the Diptera, an attempt was made to fulfill the first requirement by the selection of fixatives that most nearly preserve the appearance of the living chromosomes, while the other requirements were met by sectioning the salivary glands in a hard methacrylate matrix.

The details of structure revealed by this study indicate that fully formed giant chromosomes consist of dense bands or granules of a homogeneous substance held together by an unoriented material, lacking a chromosomal membrane or sheath. Variations in structure, both within a species and between species, suggest that giant chromosomes may be formed from or be degraded to a state showing no visible organization.

MATERIALS AND METHODS

Generalizations concerning the fine structure of the dipteran salivary gland chromosomes have heretofore been drawn from observations made on selected, well organized chromosomes (15). However, the nuclear material of the salivary gland cells may exhibit degrees of organization ranging from a complete absence of chromosomes as morphological entities to the presence of the classical, banded structures that are so familiar to all students of biology. In recognition of this, several species of Diptera were selected. *Musca domestica* and *Phormia regina* were chosen for the poorly organized condition of their salivary gland chromosomes while *Drosophila melanogaster* and *D. virilis* provided the classical type of giant chromosomes. A total of 18 separate preparations, each containing the glands of a number of larvae, were sectioned and examined in the electron microscope. An RCA electron microscope, model EMU-2A equipped with an extended range lens, was used exclusively.

Observations in a Zeiss model GF325 phase contrast microscope of glands that had been pulled out into insect Ringer's fluid revealed that relatively minor injury to any part of a gland frequently caused a slow degeneration of the cells of the entire gland. This is in agreement with the observations of other workers (4, 7). In order to avoid this effect, the glands

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studied were pulled out directly into the fixatives, in which they were allowed to remain for at least one-half hour before removing superfluous tissues. The fixatives used were chosen on the basis of several studies of fixation for optical and electron microscopy (1, 4, 6, 10, 11, 23, 25, 30) and were checked by observing their effects on the living cells in the phase contrast microscope by means of a simple chamber through which the fixatives could be passed while the tissues were under observation. One per cent osmic acid buffered to pH 7.3–7.4 with veronal acetate (25), and 10 per cent formalin, buffered to pH 7.3–7.4 with a veronal-calcium-magnesium buffer (17a) gave the best results, causing a minimum of distortion to the cells in general and no discernable change in the appearance of the chromosomes. Formalin in 10 per cent unbuffered solution caused distortion in the form of a displacement of the nucleoplasm to one side of the nucleus. In order to obtain results that could be compared to those of other workers, glands were also fixed in 45 per cent acetic acid, in spite of the fact that it was seen to produce a dramatic shrinkage of the chromosomes. A solution of 2 per cent osmic acid in distilled water usually gave good results, but occasional failures as seen in the electron microscope made it inferior to the buffered fixatives.

After fixation, the glands were washed in several changes of tap water, dehydrated in an alcohol series, and embedded in methacrylate by the methods of Newman *et al.* (21, 22). In the embedding process, partial polymerization of the methacrylate to a highly viscous condition before immersion of the monomer-impregnated glands was found to be extremely important, since extensive fragmentation of the cells resulted when this was not done. The embedded glands were sectioned on a Spencer 820 microtome, using the thermal expansion of a brass block to advance the specimen by fractions of a micron. Standard steel microtome knives, sharpened on a flat leather strop charged with diamond powder, were used for cutting the sections. The embedding matrix was removed from the sections in acetone, and they were re-embedded in a thin collodion film and mounted on 200 mesh stainless steel screens for observation in the microscope.

The sections were cut at random angles through the glands and, as a result, few nuclei appeared in any one section. In order to place the screens on the sections so that the nuclei would not be obscured by screen wires, this operation was carried out with the aid of a binocular microscope. An electromagnet mounted in a modified Emerson micromanipulator was used to place the screens so that the greatest possible number of nuclei were over screen openings (22). For each preparation, a total of about twenty sections was studied.

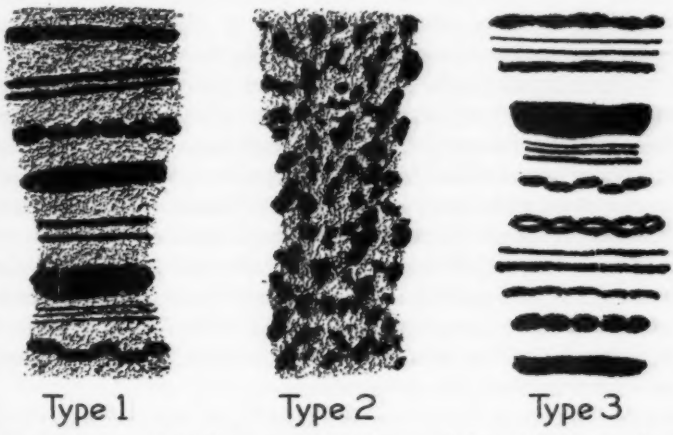
OBSERVATIONS

The giant chromosomes lie in a tangled mass like a loosely rolled ball of yarn and, as a consequence, the chromosomes in any particular thin section were cut at random angles to their long axis. Longitudinal sections through portions of chromosomes could easily be recognized by their elongated shape or by the appearance of dense cross-bands (Figs. 5 and 6).

In the two species of *Drosophila* studied, the chromosomes usually exhibited a high degree of organization, exemplified by the appearance of distinct, dense bands and by a marked contrast between the interband material and the nucleoplasm (Fig. 3). This was particularly striking in glands fixed in 45 per cent acetic acid, which caused the chromosomes to shrink and become denser and apparently extracted protein from the nucleoplasm* (Fig. 1). In a few

* Spectroscopic examination of 45 per cent acetic acid extracts of isolated dogfish erythro-

nuclei in these species, however, the interband material was almost indistinguishable from the nucleoplasm, the bands appearing to be completely disconnected, although still well oriented with respect to each other (Fig. 7). In *Musca* and *Phormia*, a well organized condition of the chromosomes was the exception rather than the rule. In these species, some nuclei did not contain any structures that could be identified as chromosomes, whereas others contained chromosomes as distinct as those seen in *Drosophila*. The possibility that such variations could be produced by the action of the fixatives was contraindicated by two observations. First, nuclei in living glands examined in the phase contrast microscope showed the same sort of variation, and second, various degrees of organization could be observed in the cells of a single gland, all of which had been fixed in an identical manner.



TEXT FIGURE. Three of the four morphological types of giant chromosomes described in the text. Since Type 4 chromosomes are dispersed in the nucleoplasm, they cannot be illustrated in this manner. See Fig. 10.

The extreme variations in the morphology of the giant chromosomes make it advisable, for simplicity of presentation, to classify them arbitrarily into the following types:

Type 1. With distinct dense cross-bands; interband material clearly distinguishable from the nucleoplasm.

Type 2. Dense material present as randomly distributed granules; chromosomes distinct from the nucleoplasm.

Type 3. With distinct dense cross-bands; interband material indistinguishable from the nucleoplasm.

cyte nuclei, done at the Marine Biological Laboratory at Woods Hole under the direction of Dr. D. Mazia, indicated that proteins were, in fact, extracted by this common fixative.

Type 4. Chromosomal material apparently diffused throughout the nucleoplasm; similar to the normal interphase condition of chromosomes.

In view of the shrinkage of the chromosomes observed in the phase contrast microscope upon application of 45 per cent acetic acid, it was not surprising to find that sections of chromosomes so treated showed a disruption of fine structure when examined in the electron microscope (Fig. 1). In addition to the shrinkage, acetic acid apparently caused the formation of an alveolar structure in the chromosomes as well as in the nucleoli and the cytoplasm. This effect has also been observed by Doyle and Metz (11, 19, 20). The alveoli in the chromosomes were frequently arranged in a manner that suggested a coarse longitudinal fibrillar structure, but similar parallel fibrils resulting from coagulation could be found in the cytoplasm (Fig. 2). Because of these artifacts, 45 per cent acetic acid was not used for studying fine structure.

The results of fixation with osmic acid or buffered formalin were so similar with respect to the chromosomes that they could hardly be distinguished. With these fixatives, several morphological features appeared to be common to all four types of organization. First, the chromosomal material appeared to be in intimate contact with the nucleoplasm. There was no evidence of a surrounding membrane or sheath. Second, the interband material seemed to be composed of fine, unoriented fibrils and granules arranged in a pattern similar to the pattern of the nucleoplasm. There was no evidence of radial symmetry in cross sections of the chromosomes (18). Third, no well-defined particulate or granular internal structure was seen within the dense material composing the bands or large granules of the chromosomes.

In the two species of *Drosophila*, the majority of nuclei contained chromosomes of Type 1 (Figs. 3, 4, 5, 6). The bands showed the variety of appearances familiar to the optical microscopist, ranging from homogeneous structures to rows of discrete granules. Though the majority of bands were sufficiently wide to be visible in the optical microscope, many were less than 0.1 μ in width. In addition to Type 1 chromosomes, some of the nuclei in these species, particularly in *D. virilis*, contained chromosomes of Type 3. Most nuclei in which this type of chromosome was found appeared to be perfectly normal (Fig. 7), but in some instances it was found in nuclei that presented a dense, pyknotic appearance, lying in cells that were literally packed with large secretion droplets (Fig. 8). Such cells have been associated with the late stages of larval development in *Drosophila* (2), and it is highly probable that the appearance of Type 3 chromosomes in pyknotic nuclei is associated with the degeneration of the gland which begins just prior to pupation.

The majority of giant chromosomes in the larval salivary glands of *Phormia regina* were of Type 2 (Fig. 8), lacking distinct cross-bands. The crossbands were also not evident when the living glands were examined in the phase contrast microscope. In the sectioned material, the chromosomes appeared to be

composed of masses of dense granules of varying size and shape, randomly distributed in a matrix of material that was structurally similar to the interband material of the giant chromosomes of *D. melanogaster*.

The chromosomes of the salivary glands of *Musca domestica* were unique inasmuch as they included all four morphological types, ranging from the classical Type 1 to the structureless Type 4 (Figs. 10, 11, 12, 13). Nuclei containing Type 4 chromosomes were similar to the nuclei which are normally found in "resting" somatic cells after fixation with formalin or osmic acid, since they were rather homogeneous or, at most, contained scattered granules and masses of varying density without any suggestion of chromosomal organization.

DISCUSSION

It is commonly accepted that the giant chromosomes of the Diptera are polytene in nature (9), that is, they arise by multiple longitudinal division of the chromonemata without subsequent separation, but attempts by numerous workers to demonstrate a longitudinal fibrillar organization have produced such conflicting results that it is extremely doubtful that it exists at all. Kodani (16), after treating chromosomes with strong alkali, concluded that the basic number of chromonemata in *Drosophila* was four. Frolova (12) determined by enzyme digestion techniques that the giant chromosomes of *Drosophila* were made up of sixteen chromonemata. Bridges and Koltzoff (3, 17), working independently, found that the chromonemata were relatively few in number and believed that they were wound spirally around the periphery of the chromosomes. Ris and Crouse (29) postulated that the giant chromosomes consisted of a number of helically coiled chromonemata, the crossbands being nothing more than an optical illusion created by the overlapping of the gyres of the helices. Metz (20) calculated, on the basis of the size of the chromosomes relative to that of normal chromosomes, that the chromonemata were extremely numerous but that they were too small to be visible in the optical microscope. He envisioned the crossbanding as being due to the alveolar nature of the chromosomal substance. Attempts to demonstrate the presence of longitudinal fibrils by means of polarized light microscopy (28), electron microscopy (13a, 26, 27, 31, 32, 33), micrurgical techniques (4, 7, 8, 13, 28) and X-ray diffraction (5) have proven to be equally confusing, and it is only in the indirect evidence offered by the cytogenetecist that we find any support for the idea that the giant chromosomes are polytene. Even so, the evidence indicates only that the crossbands are arranged in a definite, genetically controlled order (14, 24) but gives no indication of either the mechanism involved in their organization or the nature of the structural framework in which the bands lie when the chromosomes are fully formed.

The evidence presented here strongly suggests that fully formed giant chromosomes consist of homogeneous masses of dense material which may be

present either in the form of crossbands or randomly distributed granules, held together by an unoriented substance. The whole chromosome is in intimate contact with the surrounding nucleoplasm and lacks a chromosomal membrane or sheath. This is essentially in agreement with the observations of Palay and Claude (26), who used a replica method for the study of giant chromosomes of *D. melanogaster* in the electron microscope.

It is difficult, in a purely morphological study of fixed material, to draw conclusions regarding the dynamics of the formation of chromosomes. However, the variety of types of nuclear organization found in the salivary glands of *Musca* can be arranged in a sequence that can be considered as a progression of events leading to either the organization of the chromosomes from the apparently homogeneous substance of the interphase nucleus or to the disappearance of the chromosomes as morphological entities in the "resting" nucleus. Figures 10 to 13 show what may be interpreted as the transitional stages in the reorganization of the chromosomal substance in *M. domestica* from a state of complete diffusion in the nucleoplasm to the formation of the distinctly banded, completely formed giant chromosomes. Starting with the diffuse condition illustrated in Figure 10, the dense material which will eventually form the bands accumulates in large granules scattered through the nucleoplasm (Fig. 11). As the process continues, these granules form into bands which lie in parallel array in a vaguely outlined substance of slightly greater density than the nucleoplasm (Fig. 12). Finally, the chromosomal material "condenses" and forms the familiar banded chromosomes (Fig. 13). The very nature of the development of the giant chromosomes suggested here makes it highly unlikely that a chromosomal membrane could exist prior to the complete "condensation" of the chromosome. It should be noted that there is no evidence of longitudinal fibrillar organization at any stage in this process.

No attempt was made to identify genes in the giant chromosomes for a number of reasons. In the first place, the question of whether the gene has a particulate expression resolvable by the electron microscope has not been settled with any degree of certainty. In the second place, assuming that genes were of such a nature that they could be seen in the electron microscope, techniques for distinguishing them from other sub-microscopic granules and fibrils have not been developed. In the third place, it is not known whether the sub-microscopic fibrils and granules forming the "background" in sections of fixed material actually represent the true structure of protoplasm in the living cell. The visualization of the gene awaits further development of techniques in electron microscopy, as well as additional knowledge concerning the nature of the gene.

SUMMARY

Giant chromosomes of the larval salivary glands of four species of Diptera, when fixed, sectioned, and examined in the electron microscope, showed varia-

tion in structure, both within a species and between species, ranging from a distinctly banded, well organized condition to a state of complete diffusion in the nucleoplasm. The chromosomes were arbitrarily classified into four morphological types having several structural features in common. They appeared to be composed of unoriented material, lacking a limiting membrane or sheath, and contained dense material in the form of bands or large granules. The dense material, whether in the form of randomly distributed granules or well-formed cross-bands, appeared to be homogeneous. Genes could not be recognized.

On the basis of observed variations in morphology it is proposed that the classical giant chromosomes are dynamic structures. They may condense from a state of dispersion in the nucleoplasm or revert to such a state.

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PLATE I

FIG. 1. Nucleus of *D. melanogaster* fixed in 45 per cent acetic acid. The alveolar condition of the nucleolus (large central mass) and the chromosomes, as well as the apparently shrunken condition of the chromosomes, is a result of poor fixation. 5,700×.*

FIG. 2. Section of a giant chromosome of *D. melanogaster* lying next to the nuclear boundary, fixed in 45 per cent acetic acid. The alveoli produced by the fixative are arranged so that the interalveolar material forms longitudinal fibrils. Similar fibrils are present in the cytoplasm in the upper part of the picture. The nuclear membrane has been destroyed by the fixative. 22,200×.

* Unit distance on all figures equals one micron.

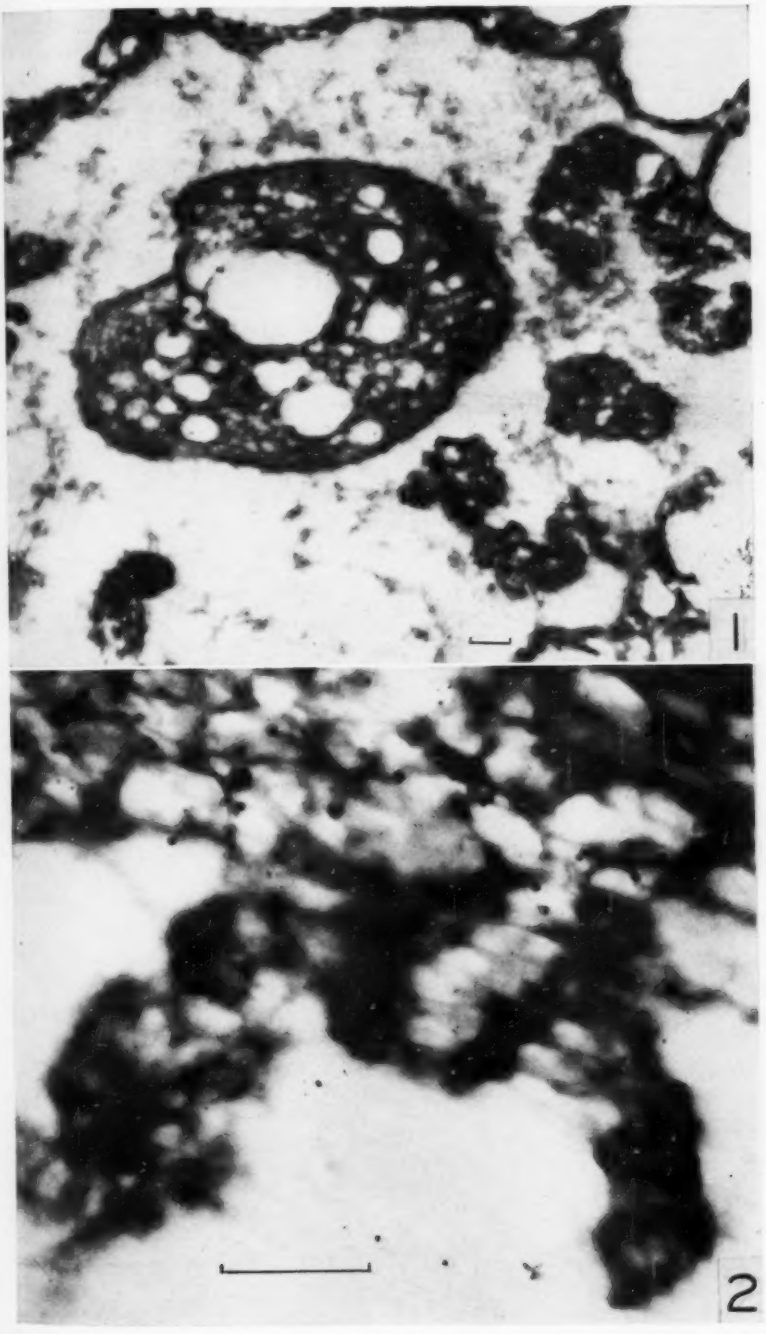
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PLATE II

FIG. 3. Nucleus of *D. melanogaster* fixed in 10 per cent buffered formalin. Type 1 chromosomes. Crossbands are distinct, interband material is unoriented and distinct from the nucleoplasm, and there is no evidence of a chromosomal membrane. The dark central body is the nucleolus. The mass of dense granules in the upper left corner is probably the chromocenter. There is a suggestion of a connection between the chromocenter and the nucleolus. The nuclear membrane has been preserved by the fixative. 5,700×.

FIG. 4. Nucleus of *D. melanogaster* fixed in 10 per cent buffered formalin. Radial symmetry is lacking in the two cross sections of chromosomes in the upper central portion of the picture. The chromosome showing a suggestion of longitudinal fibrillar organization (left of center) is shown at higher magnification in Figure 6. 5,700×.

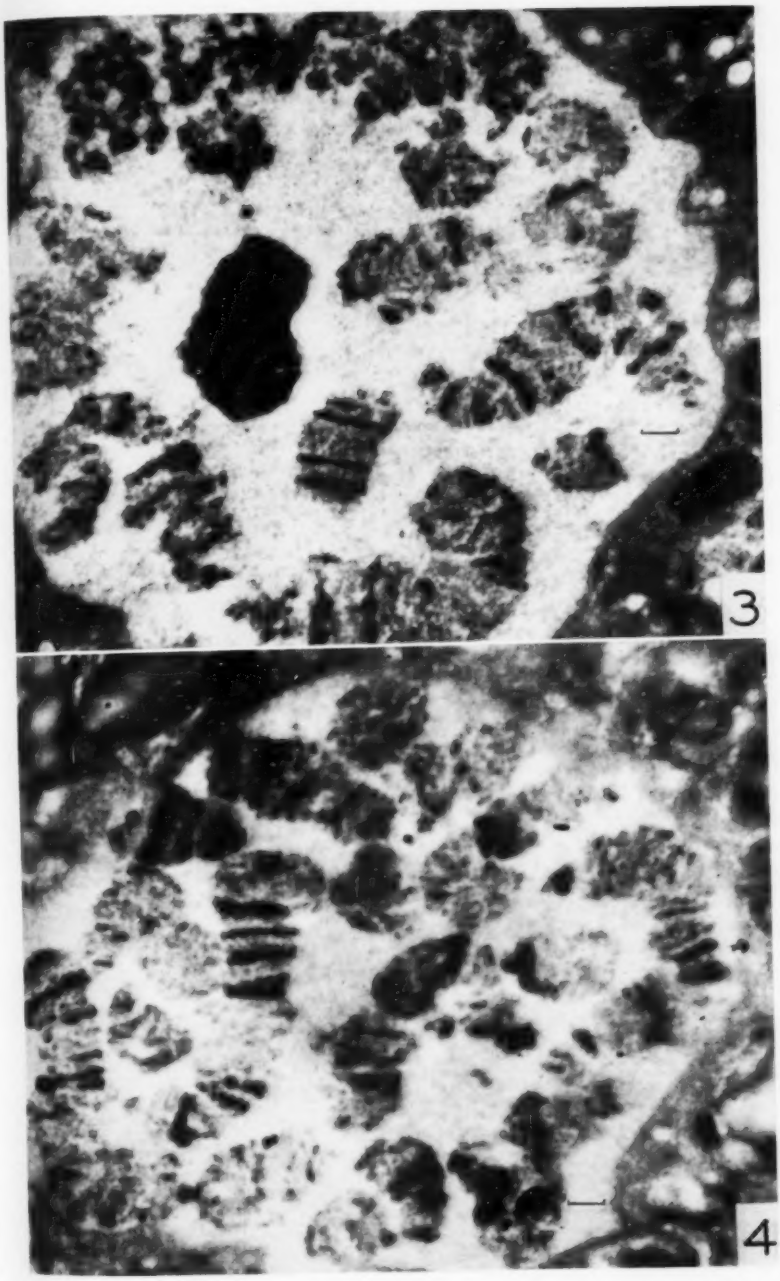
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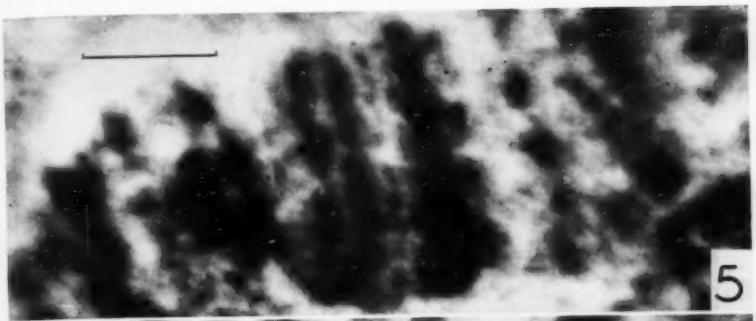
PLATE III

FIG. 5. Type 1 chromosome of *D. melanogaster*, fixed in 10 per cent buffered formalin. 21,300×.

FIG. 6. Type 1 chromosome of *D. melanogaster*, fixed in 10 per cent buffered formalin, illustrating a rare case in which fibrils appear to connect two cross bands, one of which is made up of discrete granules. In the few sections in which such fibrils were seen, they did not extend between more than two crossbands. 23,000×.

FIG. 7. Type 3 chromosome in *D. virilis*, fixed in 1 per cent buffered osmic acid. Crossbands are distinct but the interband material is not readily distinguishable from the nucleoplasm. A chromosomal membrane is lacking. The dense material of the crossbands does not possess a discrete particulate or fibrillar structure. The dark mass in the lower right corner is the nucleolus. 14,900×.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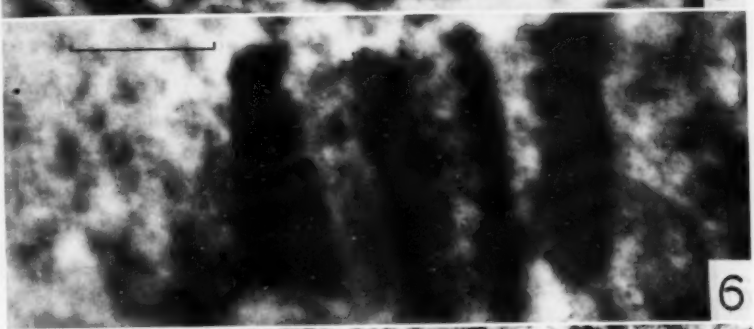
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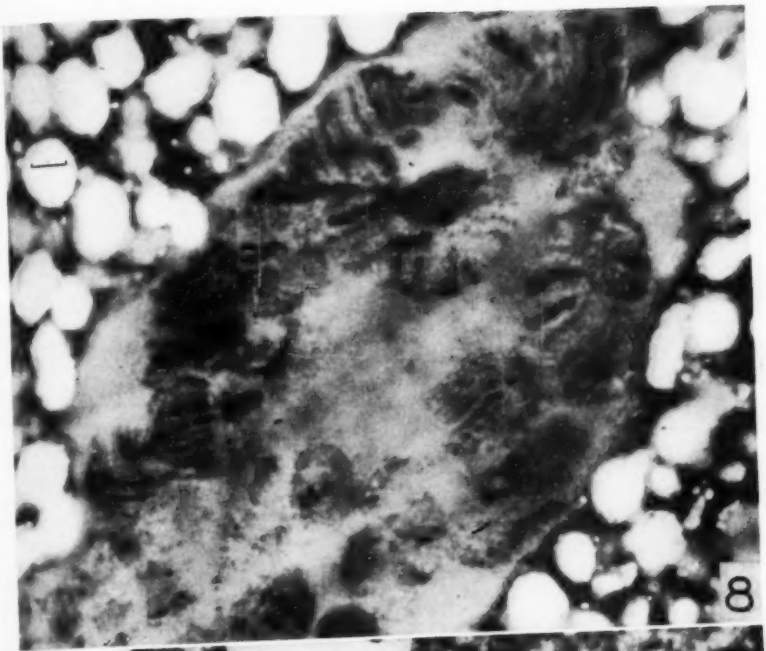
PLATE IV

FIG. 8. Type 3 chromosomes in a pyknotic nucleus of *D. virilis*, fixed in 10 per cent buffered formalin. The cytoplasm consists of a mass of large secretion droplets. The density of the nucleoplasm in this type of nucleus was so extreme that the crossbands were distinguishable only in the thinnest sections. 5,700×.

FIG. 9. Type 2 chromosomes in *P. regina*, fixed in 1 per cent buffered osmic acid. The dense material of the chromosomes is in the form of randomly distributed granules. The intergranular material is distinct from the nucleoplasm. There is no evidence of a longitudinal fibrillar organization. Chromosomal membranes are not present. The large dark granules are probably nucleolar material. 14,900×.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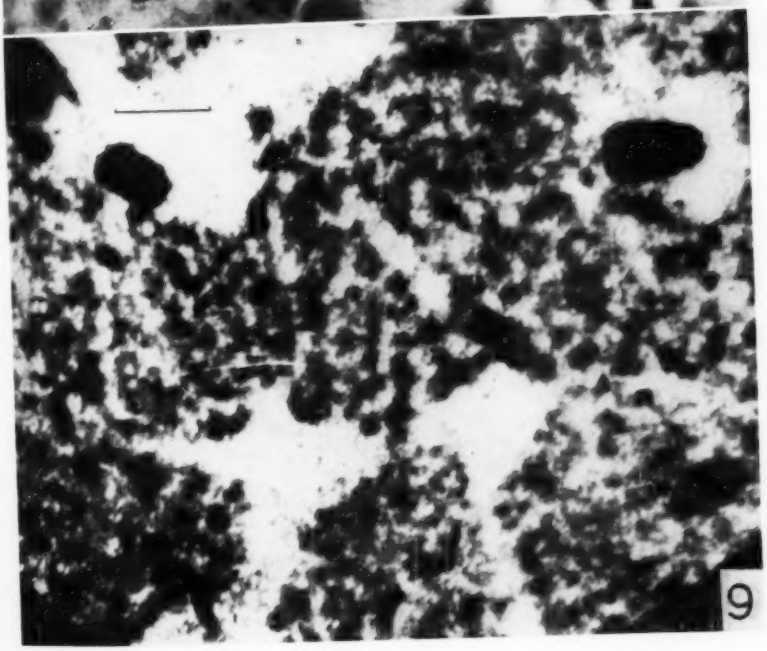
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PLATE V

FIG. 10. Nucleus of *M. domestica*, fixed in osmic acid, containing Type 4 chromosomes. The dark masses are nucleolar material. 5,700×.

FIG. 11. Type 2 chromosomes in nucleus of *M. domestica*, fixed in 1 per cent buffered osmic acid, suggesting a transition between the Type 4 condition and the Type 1 condition. 5,700×.

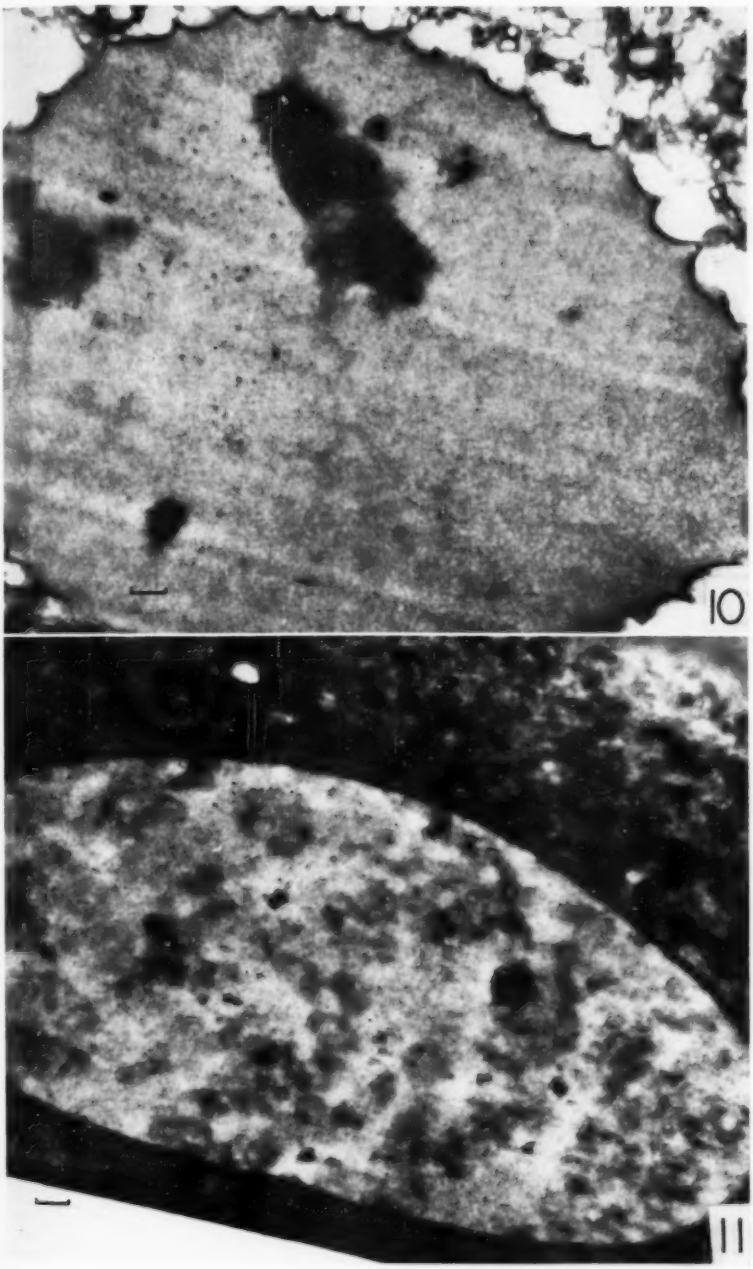
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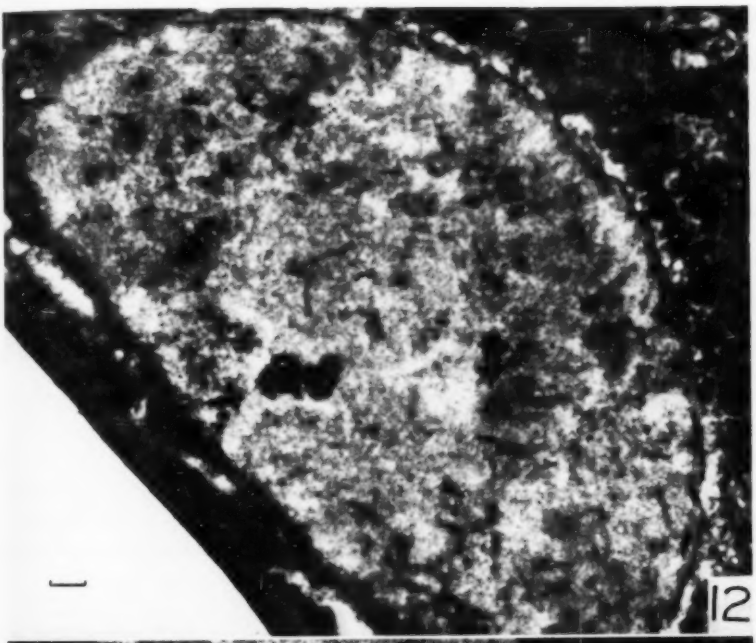
PLATE VI

FIG. 12. Nucleus of *M. domestica*, fixed in 1 per cent buffered osmic acid. The dense material of the chromosomes is arranged in bands and granules so as to suggest a transition between Type 2 and Type 1 chromosomes. 5,700×.

FIG. 13. Type 1 chromosomes in nucleus of *M. domestica*, fixed in 10 per cent buffered formalin. The dark mass is the nucleolus. 5,700×.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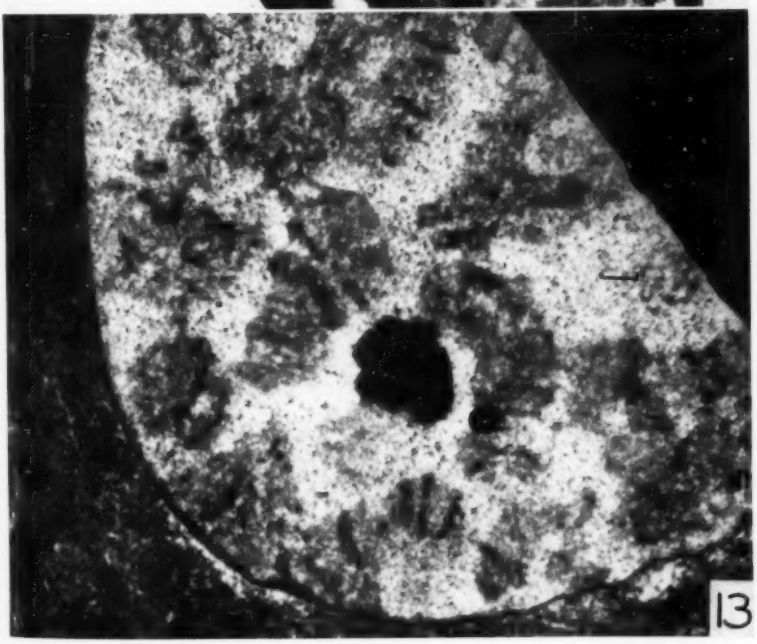
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FOUR DECADES OF MEDICAL PROGRESS*

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President Gordon,

Distinguished Guests, Members of the Graduating Class, Ladies and Gentlemen:

I count it a special privilege to participate in these graduation exercises today, for reasons which are in part entirely personal. The preceding President of this institution, Dr. Louise Pearce, and I sat in a theatre in Baltimore on a warm afternoon exactly forty years ago yesterday and were publicly awarded the degree of Doctor of Medicine by President Ira Remsen of the Johns Hopkins University. Neither of us, I am sure, ever dreamed at that time that forty years later we should be participating in another commencement ceremony, but here we are again in somewhat similar circumstances after forty years, and you graduates of today can make whatever you please out of that fact!

I, at any rate, propose to take full advantage of it, first, by making public acknowledgment of the great debt which I personally owe to Dr. Pearce for the assistance which she gave me thirty years ago when I entered a field of research in which she herself was at that time busily and productively engaged, and, secondly, by saying, as the responsible executive officer of the School from which we both received our medical degrees, how proud the Faculty of that School is to number Dr. Pearce among its distinguished graduates. The office I have the honor to hold at the present time gives me the right to make that statement and under that authority I make it here and now with the greatest pleasure.

I also propose to take advantage of the fact that Dr. Pearce and I are forty years old, medically speaking, by using it as the basis for this address. I propose to tell you graduates, who will in a few weeks or months begin your hospital internships, something of what Dr. Pearce and I, starting as interns on the same medical service forty years ago, had to do as house officers, what sort of problems we had to face, and some of the tools with which we had to work. I do this because I want you to understand how far medicine has come in these last four decades, and how hard you of the younger generation will have to work, if medicine during the next forty years is to develop as rapidly and to as great an extent as it has during the past forty years.

I would not be greatly surprised if future medical historians, looking at the period covered by the first half of the present century, should refer to it as a

* Delivered at the Commencement Exercises of the Woman's Medical College of Pennsylvania, Philadelphia, Pennsylvania, June 12th, 1952.

period during which the greatest strides were made in the control of the acute infectious diseases, and in the development of many new methods for the treatment of illness. Certainly the progress in these two directions during the past forty years, at least, has been breathtaking.

On the one hand we have seen many of the most serious infectious diseases almost wiped out, either through the introduction of sound sanitary measures on a mass basis, or through the use of newly-discovered drugs of great potency; on the other hand, through gaining a better understanding of how the body works we have seen the employment of new agents in the treatment of certain chronic diseases which have completely revolutionized the outlook for patients who are suffering from those illnesses. Let us look first at some of the acute infectious diseases.

When Dr. Pearce and I reported for duty on the Medical Service of the Johns Hopkins Hospital on September 1st, 1912, we were assigned to the public wards and there we found that nearly one-half of the patients for whom we were responsible were in the various stages of typhoid fever. Some were at the height of the disease, some were defervescing, some were up and about, and some were even starting on a second attack of the disease!

In that year of our internship there were 120 patients with typhoid fever admitted to the Johns Hopkins Hospital and seven of them died. We had no specific drug to give them. Treatment consisted of bathing the patient every four hours day and night so long as his temperature reached 102.6° Fahrenheit. A tub was trundled into the ward alongside the bed and the patient was lifted into it by a nurse and an orderly, after which he was massaged by the nurse for fifteen minutes while he lay in the tub, his teeth chattering all the while. How the nurses managed to stand up under such back-breaking work I will never know. More than one of them contracted the disease in line of duty. This type of treatment, we were told, reduced the mortality of the infection but I have often wondered whether the figures brought forward in support of that statement would have withstood a modern statistical analysis.

Now the situation is completely changed. In the Johns Hopkins Hospital last year there were only two cases of typhoid fever and in all Baltimore only five, with no deaths, whereas in 1912, when the city was only half as large as it is now, there were over a thousand cases. Indeed, I wonder if any of you members of the graduating class have ever seen a typhoid rose spot? Personally, I hope that you will never have the opportunity to see one, for we can well do without typhoid fever in this country of ours. Its almost complete eradication we owe to improved sanitation and to some extent to immunization, and not to the development of any specific drug for it.

For us interns in 1912 lobar pneumonia was a dreaded disease. The case-mortality rate in the Johns Hopkins Hospital during our intern year ranged around twenty-five per cent. We had no specific drug or biological agent with

which to treat our pneumonia patients. About all that we could do was to see that they got plenty of fresh air and were made as comfortable as possible while we waited anxiously to see if they would recover. It is true that we may have given them what we fondly hoped were supportive drugs in the form of strychnine or caffeine or camphorated oil, but as I look back on that year I wonder if we really saved any lives by what we did. Now, however, the whole aspect of the treatment of acute lobar pneumonia has changed, thanks to the newer drugs that we call antibiotics. Recovery from pneumonia within 24 to 48 hours is a commonplace, and one almost never hears any more about the "crisis" in that disease.

Malaria was no stranger to us in the Johns Hopkins Hospital in 1912. There were thirty-three cases of that disease on the medical wards that year. In all Baltimore last year there was only one case of malaria reported. Unless you elect to practice in the tropics you graduates are not likely to have much of an opportunity to see malarial patients but that is all to the good in my opinion.

While epidemic meningitis was not particularly prevalent in 1912 it was always greatly dreaded because of its high case-mortality rate which not even Flexner's anti-serum could altogether eliminate. Now, thanks to the newer drugs, this particular disease no longer alarms us as much as it did in the past.

This conquest of some of the acute communicable diseases can best be appreciated if we take a brief glance at the records of the health departments of any of our larger cities. I will give you the figures for Baltimore, for which I am indebted to Mr. Matthew Taback, Director of the Bureau of Biostatistics of the Department of Health of that city, merely because they were easily accessible to me. I have no doubt that they offer a reasonably good sample of what has been taking place elsewhere in the country.

The death rate from five communicable diseases in Baltimore, namely typhoid fever, diphtheria, whooping cough, measles and scarlet fever, taken together, dropped from 80.6 per 100,000 of population in 1910 to 0.8 per 100,000 of population in 1948, or one one-hundredth of what it was forty years previously. To put it in another way, these five communicable diseases in 1910 were responsible for 4.2 per cent of all the deaths in the city, whereas in 1949 they accounted for only one-tenth of one per cent of all the deaths.

Pneumonia and influenza together accounted for 207 deaths per 100,000 population in Baltimore in 1910; in 1949 they accounted for only 32 and a fraction deaths per 100,000 population. Diarrhea and enteritis in children under two years of age, "summer complaint" as it was called by the older generation, brought about 107 deaths per 100,000 population in Baltimore in 1910; in 1949 the corresponding figure was only 5, or one-twentieth of what it was at the earlier date.

I fear that it is only we medical people of the older generation, who have witnessed this change, who are really in a position to appreciate what has

happened in the country during the past forty years in respect to the mortality from acute infections, but I would remind you that thus far I have been talking principally about the frequency of deaths from these diseases, not about the frequency with which some of them occur in the community. Unhappily we still meet all too frequently with the common cold, whooping cough, scarlet fever and streptococcic sore throat, measles and infantile paralysis. We must still try to eliminate them altogether.

Let us now take a brief look at two of the chronic infections. Everyone knows that the death rate from tuberculosis has been declining for many years. This decline has been striking within the last forty years. In Baltimore, where there is still altogether too much tuberculosis, the death rate from all forms of this disease has declined from 250 per 100,000 of population in 1910 to 63.3 per 100,000 in 1949, a reduction to one-fourth of what it was during the earlier year. This decline is unquestionably due to a number of different factors and I think that it would be rash to attempt to ascribe to each individual factor its precise role, but there is no doubt that in very recent years the antibiotic streptomycin in combination with other drugs has brought about a sharp reduction in the mortality rate of at least one type of tuberculosis which for many years was uniformly fatal. I refer to tuberculous meningitis.

In 1912 there were eight cases of this disease treated on the wards of the Johns Hopkins Hospital. All of them died. They might just as well not have been admitted to the hospital for all that we could do for them at that time. Now the situation is by no means as dark as it was. In the last two years ten consecutive cases of tuberculous meningitis have been treated in the same hospital with streptomycin and para-amino-salicylic acid, and 8 of them have recovered. Moreover we have a still newer drug, which looks promising for the treatment of this disease, namely, isonicotinic acid hydrazid, or isoniazid, as it is now called. This is the drug which received so much premature publicity this past spring. Last month at Atlantic City Dr. Walsh McDermott of Cornell University Medical College reported on a series of cases of tuberculous meningitis treated with this drug alone or in combination with streptomycin. The results were encouraging, to say the least, and certainly more favorable than would have been the case had the patients received no treatment at all. However, we must not jump too quickly to conclusions as to what this new drug can do. Much more study will be necessary to determine the degree of its effectiveness in all forms of tuberculosis but for the moment, at any rate, it looks promising and it is just possible that we are on the threshold of another important step forward in our long struggle against this arch enemy of mankind.

Another chronic infection, syphillis, appears to be on its way out, thanks chiefly to penicillin and more effective measures to discover and bring patients with this disease under treatment, but the elimination of this infection is a most difficult problem because of its social implications. It is a great pity that we

have not as yet learned how to immunize human beings against this, the great pox, as it was once called, as we have learned how to immunize them against the small pox.

Let us leave the acute and chronic infectious diseases at this point and turn to those which represent some basic fault in the chemistry of the body. Here the story of what has happened during the last forty years is no less thrilling than that of the diseases due to microbial agents. In the time at my disposal I can point to only a few high lights in this field.

In 1912 there were 27 patients with pernicious anemia admitted to the medical wards of the Johns Hopkins Hospital. Three of these patients died. There is no excuse nowadays for any patient with pernicious anemia to die of that condition alone, thanks to the work of Whipple and Minot and Murphy. Indeed, so well do these patients respond to treatment with liver extract or the more refined preparations of vitamin B_{12}, that they may be safely treated in the doctor's office and do not need to enter a hospital for a prolonged stay as was the case when Dr. Pearce and I were interns.

A similar situation prevails in respect to diabetes mellitus. Forty years ago all that we interns could hope to do for our diabetic patients was to try to teach them how to control their diets but that was up-hill business at best, and of course particularly difficult in the case of children. In fact, children with diabetes in those days almost never lived more than a few years. Then came the discovery of insulin and the whole picture changed, most and best of all insofar as the children were concerned. Now the diabetic child can expect to grow up to adulthood, and can lead a normal active life.

Finally there has recently come cortisone and with it ACTH, newest of the preparations which we call hormones, of which I am sure that everyone in this audience has either read or heard. These new arrivals have raised high hopes that we may be able to control some of the crippling forms of chronic rheumatism and other conditions as well, although again we need to reserve judgment as to their ultimate value in the treatment of disease. Nevertheless they have already opened up important avenues of investigation and promise to yield much fundamental information about the normal behavior of the body in addition to the benefits which they confer in the actual treatment of patients.

When we consider all of these great advances that have been made in the treatment of illness during the last forty years is it any wonder that we presume to speak of such a thing as a therapeutic renaissance during that period? This renaissance may perhaps best be appreciated by you graduates of today if I tell you something about the tools with which we graduates of 1912 had to work when we started out as medical interns in that year.

In the text book of pharmacology which Dr. Pearce and I studied, the drugs then available were classified according to their use. In the class labeled "Drugs used for specified diseases", there were only five diseases listed, in other words

only five for which there were considered to be specific drugs available at that time. These were malaria, for which quinine and arsenic were listed; syphilis, for which there was mercury and iodides; rheumatic fever, for which there were the salicylates; myxedema or thyroid deficiency for which thyroid extract was available; and gout, for which there was colchicum. Of course there was digitalis for a failing heart, and drugs that could stimulate or depress the functions of various parts of the body, or induce sleep or abolish pain, and there were drugs that would rid the intestinal tract of worms, or remove parasites from the external layers of the skin, but specific chemotherapeutic agents in the sense in which Paul Ehrlich used the term, that is, agents that would attack a specific microbe that produced a particular disease, and destroy it, were lamentably few in number. Indeed, Ehrlich's own great contribution to the treatment of syphilis, salvarsan, was only just beginning to be used in this country when we graduated.

At that time we knew almost nothing about the so-called food deficiency diseases except the fact that fruit juice would prevent scurvy and that there was a curious disease in the Orient called beri-beri that some investigators thought might be due to lack of something in the diet but others were convinced was due to eating spoiled fish. The text book on pharmacology, to which I have already referred, had this to say about cod-liver oil:—"Its effects are obviously those of an easily assimilable food, and it is not a drug in the ordinary sense of the term, and has therefore no place in pharmacology properly speaking, but should be classed along with other foods"! You will perhaps better understand the reason for that statement when I tell you that at the time that particular edition of that text book appeared the term "vitamin" had not yet been coined, nor had it been shown that cod-liver oil contains a vitamin that prevents rickets in children. Nowadays every young mother knows that her baby should receive cod-liver oil as a protection against the bone deformities of rickets, and the number of vitamins which have been discovered since 1911, when the term was first introduced, is already sufficiently great to tax the sources of the alphabet whose letters are used to label them.

Transfusion was for us interns a comparatively rare form of treatment, carried out in the operating room by the cumbersome direct method of connecting an artery of the donor with a vein of the patient, and it was several years after our graduation before the present indirect method using citrated blood was introduced. Now there are blood banks all over the country in which blood is kept on deposit to be used for the next patient who needs a transfusion, and we collect blood from human beings and from it make dried plasma which we ship to remote parts of the earth for the benefit of our soldiers wherever they may be engaged in fighting.

Thus far I have been talking only about the treatment tools we medical interns of 1912 had to work with, in comparison with those which are available

to the medical intern of today. What of the diagnostic tools which were available then, compared with those which are at your service now?

We had the stethoscope and the microscope, of course, and rather primitive ophthalmoscopes and otoscopes with which it was somewhat difficult for the novice to see the retina or the tympanic membrane, and of course we could measure the blood pressure and take recordings of the pulse with Sir James Mackenzie's polygraph if we had the time, which we usually did not, but we were as yet quite unfamiliar with the uses and the limitations of the electrocardiograph, which was just beginning to be used in the clinic, and as for the electroencephalograph, that reveals the so-called "brain waves", it had still to be invented.

The X-ray was available, of course, but it had not yet been used in connection with chemical substances to make the gall bladder visible, nor, with the aid of air injected into the ventricles of the brain, had it yet begun to play an important role in the diagnosis of brain tumors.

We had of course learned to count the blood and to perform the simpler chemical tests on the patient's excreta, but as yet, except for somewhat crude methods for the determination of the hemoglobin content of the blood, there were practically no methods for making accurate quantitative determinations, in the clinic, of this, that or the other chemical substance present in the blood, determinations which are so helpful in finding out what is wrong with the patient and the extent of the trouble, and which can be made nowadays in relation to a great many components of the blood. All of the advance in this particular direction we owe to the remarkable development of accurate chemical analytical methods applicable to the fluids and tissues of the body, which has taken place in the last few decades.

Similarly, we had few methods for testing the various functions of the body or of its special organs. The phenolsulphonephthalein test of Rowntree and Geraghty for kidney function had just been introduced (1910) but there were no satisfactory tests for liver function as yet, and the determination of the basal metabolic rate, so important from the standpoint of the management of the patient with thyroid disease, had yet to find its way into the clinic.

Only on the bacteriological side may we be said to have been not too badly off in 1912 for diagnostic tools, thanks to the great advances made in bacteriology during the previous century. We could isolate and cultivate many of the disease-producing microorganisms from our patients, even from their blood, for diagnostic purposes, and we could examine the patient's blood for the presence of antibodies against certain diseases. Likewise we had learned to inoculate the smaller laboratory animals with material from the patient and make a diagnosis by recovering a disease-producing microorganism from the inoculated animal, just as you can today. Looking back at those intern days, however, I would have to admit that our diagnostic tools were distinctly

limited compared with those which are available to you graduates today. Indeed I think that the advances which have taken place in respect to our diagnostic armamentarium are perhaps as great as those which have occurred in relation to our treatment armamentarium, although perhaps not as dramatic.

I have not had the time, in this brief and highly fragmentary survey of medical progress during the forty years of my professional life, to cover all of the brilliant achievements that have come to pass in the field of medicine. I have had perforce to neglect what has happened in surgery, obstetrics and the many special fields, and have been obliged to concentrate largely upon what has happened in internal medicine, and, to some extent, in the field of public health. To have attempted to cover the progress that has been made in all of the clinical and pre-clinical fields during the past forty years would have prolonged this address far beyond the point of human endurance, even if it were possible for one human being to cover all those fields adequately, which is not the case. But I think that the facts which I have laid before you offer sufficient evidence for the claim that during these last four decades really startling progress has been made in the field of medicine and justify my raising with you young people at this point, two questions. They are:—What has all this progress of the past forty years led to in terms of what you graduates will have to face in the future, if you elect to enter the practice of medicine? And what will *you* do to contribute to medical progress in the next forty years during the active period of *your* professional careers?

The first and obvious answer to the first of my two questions is that there are some diseases which you are not likely ever to be called upon to treat. There are others which, should you encounter them, you will be better able to handle than Dr. Pearce and I were during our intern year. These very facts, however, mean that in some respects your treatment problems will be more difficult than ours were. Perhaps I should put it a little differently and say that you will have many more of the more difficult treatment problems than we had, simply due to the fact that the patient population with which you will have to deal, will be an older population on the average, unless of course you confine your practice to obstetrics and pediatrics, for it is we old folks that present our doctors with the most difficult problems of all.

Everyone knows that nowadays human beings at birth may expect to live much longer than their grandparents had a right to expect when they were born. This increased life expectancy of the average human being in this country, which has grown from about 50 years in 1900 to almost the Biblical span of three score years and ten in 1950, means that if you enter the general practice of medicine you will find that many of your patients will be elderly people who will come to you with illnesses for the treatment of which you are not too well equipped as yet, and for which you will need all the diagnostic and therapeutic skill which you are able to muster.

You will have to think hard to solve the problems presented by those patients, and you will need always to remember that after all there is no such thing as a "disease", using the word in the sense of a finite object; there is only a patient, a human being like yourselves, who is out of sorts for one cause or another. Those causes may range all the way from the presence of an invisible agent, which we call a virus, within the patient's body, to an obnoxious in-law within the family circle. Either of these may be just as poisonous as the other!

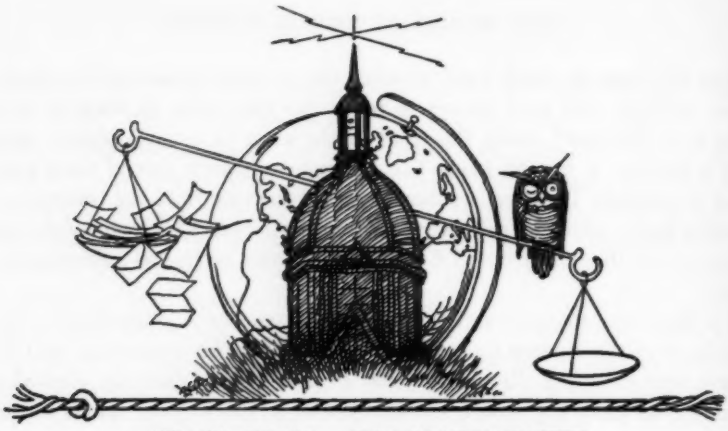
For the proper management of these future patients of yours do not rely too heavily upon the modern tools with which you have been equipped and which I have been extolling. Remember that a good history carefully elicited and, what is even more important, mulled over at length in your mind, will often give you the key to the problem that no amount of blood chemistry studies can ever reveal.

Do not forget also, that these patients of yours will not be isolated individuals, as a rule, but they will be members of a group, first the family and later, other groups of different kinds, and that that fact will often have a bearing upon what you will have to do in the management of the case.

Above all, never forget that these patients of yours, whether they seek you out themselves or whether they are sent you by another physician, have placed upon you the greatest responsibility which can be placed upon any human being, namely, the responsibility of life and death. Many persons during these past four years have labored together to train and equip you to discharge this great responsibility which you assume this day. I am confident that you will discharge it in an altogether creditable fashion, that you will not fail to justify the confidence that the faculty of this, your alma mater medica, has placed in you.

The answer to my second question, what will you do to contribute to the progress of medicine during the next forty years, the active period of your professional careers, I cannot give. That lies with each and everyone of you individually. But, if I were asked to make just one graduation wish for all of you it would be that each one of you might have the satisfaction of contributing at least one thing to the progress of medical knowledge, for in the progress of that knowledge lies, in my opinion, the greatest hope for the salvation of mankind.

And now, may I salute you, congratulate you and wish you well.



BOOK REVIEW

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Dynamic Aspects of Biochemistry, 2nd edition. By ERNEST BALDWIN. 544 pp., $5.00. Cambridge University Press, New York.

This book does not fill the most urgent need of a textbook in biochemistry but appears adapted to those who wish to read a coherent story about some of the various conclusions and concepts advanced in this field today. Students and workers in other fields might enjoy its somewhat arbitrary description of chemical mechanisms in biology. The English is excellent and the material is presented in an interesting fashion. One might learn much about possible interpretations of biochemistry from this very readable book.

However, little is presented very critically. The turbulent problems which encompass most areas of biochemical knowledge are largely avoided in this fairly serene picture, which is also little aided by quantitative data. As examples, the following statements may be found:

"It seems probable, however, that the primary reaction in transamination consists in the transference of amino-group from an amino-acid to the prosthetic group of the enzyme, pyridoxal phosphate" (p. 139).

"But, as we know today, new energy-rich bonds arise in the course of many katabolic trans-formations, and indeed, it would appear that katabolism as a whole is directed above all to the generation of new energy-rich phosphate bonds" (p. 75).

"It is significant, however, that newly generated energy-rich bonds cannot be utilized directly for the performance of endergonic operations: they can be put to service only through the intermediation of the ADP–ATP system" (p. 75).

"Enzymes are complex, organic catalysts of high molecular weight, produced by living cells but capable of acting independently of the cells that produce them" (p. 23).

"In consequence, a part of the enzyme is held in the form of enzyme-inhibitor complex, and so is not available for the catalysis of succinate oxidation, and the reaction velocity accordingly diminishes when the inhibitor is added" (p. 35).

Subjects discussed comprise many areas of biochemical knowledge but some subjects are not taken up in a complete way. Thus the synthesis of uric acid, p. 324, is discussed in

unusual detail from the standpoint of the older literature, which is notably confusing, while more recent findings which actually do illuminate the subject considerably are dismissed in one curtailed paragraph on p. 327.

FREDERICK W. BARNES, JR.

Viral and Rickettsial Infections of Man, 2nd edition. By THOMAS M. RIVERS. 719 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

In the four short years since the first edition of this book has been published, it has become the friend of beginning student, graduate virologist and clinician alike. This is no mean accomplishment. There are many reasons for this. Despite the separate and authoritative authorship of the thirty-nine different chapters, they all read well and smoothly. There is enough of the "fundamental" (techniques of examination of the viruses, multiplication of bacteriophage, etc.) so that the beginner can understand the derivation of many of the standard concepts of virology. The illustrations are profuse and excellent. Finally, the cost is minimal for a book of this kind.

This edition adds a number of important new chapters, perhaps the most useful of which is "Diagnosis of Viral and Rickettsial Infections." With all of this to commend it the reader may be irritated by references in the text which do not appear in the bibliography and by certain omissions, such as a failure to mention filamentous forms of influenza virus.

Under Dr. Rivers' able leadership this book is heavily weighted by contributors who either are now at the Rockefeller Institute or have "graduated" therefrom. This is natural, for virus research owes much to this Institute. However, very little of the approach of the comparative pathology of Theobald Smith is apparent. This is in startling contrast to the frontispiece of the book itself. Of eight viruses shown in their chrome coated splendor, three are plant viruses, three are bacteriophage, one is Shope's rabbit papilloma and only two are of human origin (vaccinia and influenza). Despite this, it is, as the title states, a textbook of the infections of man. As such it is outstanding.

FREDERIK B. BANG

Electrocardiography in Practice, 3rd edition. By ASHTON GRAYBIEL, PAUL D. WHITE, LOUISE WHEELER AND CONGER WILLIAMS. 378 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

The format of G. aybiel and White's *Electrocardiography in Practice* has had a face-lifting. The familiar elongated shape of the 1941 and 1946 editions has given away to more conventional 8 by 11 inch dimensions. There is much that is the same as in the previous editions and some significant changes; both deserve evaluation. Unlike most medical books which are implicitly or expressly intended for both student and practitioner, this volume according to the statement of the authors, is not meant to be a textbook but rather an atlas for the practitioner. Electrocardiographic pedagogy is the subject of considerable discussion. For the school which insists that electrocardiography is sufficiently advanced as a science to make it possible and desirable to familiarize the beginner with the unipolar leads and the vectorcardiogram in full detail before passing to a study of the classical leads, this is indeed not a textbook. However, those who feel that electrocardiographic interpretation is still a matter of "enlightened empiricism" can recommend this book to beginners with only minor reservations.

An admirable feature of this edition is a long introductory section in which the physiologic principles of electrocardiography are discussed in the historical sequence of the development of our knowledge about this subject. This is a good history of electrocardiography. In deference to the scientific school of electrocardiographic teaching, the authors give a simple

exposition of vectorcardiography and the unipolar leads before the limb leads are discussed. The sets of electrocardiograms illustrating various abnormalities are well chosen. What makes them particularly instructive is the well written clinical case abstract that accompanies each. Not only do these abstracts point up the frequently forgotten importance of considering the electrocardiogram in clinical context but the dramatic clinical features of each case serve to fix the electrocardiographic findings in mind. The section on normal variations displays the interests of the senior author. The sections on disturbances of rhythm and of conduction—the classical forte of electrocardiography—are excellent. (The authors deplore the increasing tendency to neglect intensive training in electrocardiographic interpretation of disorders of rhythm.) The comments introductory to the section on myocardial infarction are somewhat weak. The patterns associated with infarction of various portions of the ventricular wall are well illustrated by specific examples but one cannot help but feel that the reader would find it easier to associate the pattern with the location of the infarction if a clearer, straight-forward description of the form and lead projection of the "primary" and "reciprocal" changes in the case of an idealized infarct was provided. With this relatively minor reservation the reviewer does not hesitate to recommend this atlas to rank novices in electrocardiography and certainly to practitioners.

VICTOR A. MCKUSICK

Diseases of the Nervous System in Infancy, Childhood and Adolescence, 3rd edition. By FRANK R. FORD. 1181 pp., $18.50. Charles C Thomas, Springfield, Illinois.

The original publication of Dr. Ford's book fifteen years ago met immediate widespread acceptance. It offered pediatricians and clinical neurologists, for the first time, a comprehensive descriptive textbook of the disorders of the nervous system encountered in the early age groups. More than that, however, its scope clearly extended beyond the indicated years, and the author drew on his wide experience to describe in a lucid fashion all but the most obviously geriatric of clinical neurological disturbances.

There were several characteristic features of the book. Perhaps most important was the liberal use of illustrative case histories drawn from personal records and the files of this hospital. Mere general description of clinical features in any field of medicine often lacks clarity for the inquiring reader; important and differential points may be obscured; sequence of investigative procedures is often lacking. The extent to which these difficulties can be met by representative reports of actual cases was witnessed by this volume. Secondly the author resisted the strong temptation to introduce, in a clinical textbook, digressions into neurophysiological studies. Such digressions, sometimes incorrectly presented and frequently irrelevant, have, in the reviewer's opinion, detracted from the value and authority of certain other modern medical texts. Dr. Ford's book is, as stated, a comprehensive and sound compendium of descriptive neurology, based on extensive clinical experience.

The present edition has been completely revised, new sections added, illustrations revised and increased. There are a few errors in typography and reference, but they are infrequent. Certain minor questions might be directed towards the incidence and factors involved in Little's disease, but they are, from a practical standpoint, immaterial. The same can be said for other minor criticisms one might level. It is a pleasure to recommend highly this important textbook of diseases of the nervous system.

J. W. M.

Pharmacology in Clinical Practice. By HARRY BECKMAN. 839 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa.

In the preface to this treatise Dr. Beckman criticizes the traditional pre-clinical course in pharmacology on the grounds that the clinician does not reason forward from the drug to the patient but backward from the patient to the drug. He has attempted to remedy the

situation by discussing drugs in connection with specific diseases and symptoms which arise in medicine, the various medical specialties, surgery and dentistry. Thus, except for short chapters on anesthetics, antibiotics and autonomic drugs, the book consists of brief descriptions of most of the maladies which plague man and some fairly detailed therapeutic procedures which are used to combat them. The student is introduced to drugs by trade names and must refer to an appendix for their chemical structures or official status.

There are a number of objections to subjugating pharmacology too rigorously to therapeutics. The student does not get a clear conception of the scope of pharmacology or what type of information he may expect to obtain from a pharmacologist. He is likely to lose sight of the numerous activities possessed by most drugs and the compromises which necessarily attend their various clinical applications. The distinction between drugs of proved and unproved therapeutic value may be obscured. Finally, the amount of information which must be learned by rote is increased.

Most of the more specific objections to Dr. Beckman's book result from the obvious impossibility of his being competent in all of the many fields he discusses. Although much too thin in pharmacological fact, it is fraught with errors, some of a fundamental nature. Many of the therapeutic procedures discussed in detail are of highly questionable value. The author is unduly concerned with combinations of drugs. In addition, the book appears to have been dictated and contains several ambiguous sentences and numerous wisecracks and digressions which serve no other purpose than to annoy the reader.

It is improbable that many pharmacology departments will adopt this textbook.

E. W. MAYNERT

History of the Royal Medical Society, 1737–1937. By JAMES GRAY. Edited by DOUGLAS GUTHRIE, with a foreword by SIR ROBERT HUTCHISON. 355 pp., 42/ net. Edinburgh University Press, Edinburgh, Scotland.

This is a history of what is probably one of the oldest medical societies in the English-speaking world, the Royal Medical Society of Edinburgh. Because of its close association with the Edinburgh Medical School this is virtually a history of both institutions. The reader shares the experience of Osler (p. 269): "Looking over the list of members since 1737 I was prepared, of course, to find the names of many of the great men of the profession but I did not expect to find a list of such extraordinary distinction." Part of the charm of this history derives from the fact that it is written to a large extent as the story of the men who were members of the Society as students in Edinburgh and who played influential roles in medicine there and elsewhere. To name a few would do injustice to the many who cannot be mentioned in the limits of this note. Founded by students, this organization was unique in its student control and participation. American readers will be interested in the accounts of students from this side of the Atlantic who brought back the Edinburgh influence—John Morgan, Benjamin Rush, Caspar Wistar, and others. All will enjoy reading about the student days of those who forsook medicine for other fields—Oliver Goldsmith, Charles Darwin, and so on. The book is readable. A detailed index increases its usefulness as a source book in medical history.

VICTOR A. MCKUSICK

BOOKS RECEIVED FOR REVIEW

Bacterial and Mycotic Infections of Man, 2nd edition. Edited by RENE J. DUBOS. 886 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

Die Frühdiagnose des Uteruscarcinoms. Histologie, Kolposkopie, Cytologie, Biochemische Methoden. By HANS LIMBURG. 208 pp., Ganzleinen DM 19.50. Georg Thieme Verlag, Stuttgart, Germany.

Diseases of Metabolism, 3rd edition. Edited by GARFIELD G. DUNCAN. 1179 pp., $15.00. W. B. Saunders Co., Philadelphia, Pa.

Essentials of Body Mechanics in Health and Disease, 5th edition. By JOEL E. GOLDTHWAIT, LLOYD T. BROWN, LORING T. SWAIM AND JOHN G. KUHNS. 356 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa.

Gynäkologische Strahlentherapie. Indikationsstellung, Methodik und Ärtzliche Betreuung. By RICHARD KURT KEPP. 232 pp., Ganzleinen DM 26. Georg Thieme Verlag, Stuttgart, Germany.

The History of American Epidemiology. By C.-E. A. WINSLOW, WILSON G. SMILLIE, JAMES A. DOULL AND JOHN E. GORDON. Edited by FRANKLIN H. TOP. 190 pp., $4.75. The C. V. Mosby Co., St. Louis, Missouri.

Lehrbuch der Röntgenologischen Differentialdiagnostik. Vol. 1. By WERNER TESCHENDORF. 954 pp., Ganzleinen DM 129. Georg Thieme Verlag, Stuttgart, Germany.

Nutrition and Diet in Health and Disease, 6th edition. By JAMES S. MCLESTER AND WILLIAM J. DARBY. 710 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

Physiologie Normale et Pathologique du Metabolisme de L'eau. By JEAN HAMBURGER AND GEORGES MATHE. 502 pp., 2.850 francs. Editions Medicales Flammarion, Paris, France.

Practical Dermatology. For Medical Students and General Practitioners. By GEORGE M. LEWIS. 328 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

Rheumatic Diseases. Diagnosis and Treatment. By EUGENE F. TRAUT. 942 pp., $20.00. The C. V. Mosby Co., St. Louis, Missouri.

Röntgenuroskopie. Nierenbecken und-Kelche Harnleiter, Blase, Harnröhre. By G. W. GÜNTHER. 45 pp., Kartoniert DM 13.50. Georg Thieme Verlag, Stuttgart, Germany.

Surgery of the Eye, 3rd edition. By MEYER WIENER AND HAROLD SCHEIE. 449 pp., $15.00. Grune & Stratton, New York.